CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 1 SEPTEMBER, 1941 NUMBER 9

The Production of Cancer by Some New Chemical Compounds*

Factors Affecting the Latent Period of Tumor Production

J.T. Bradbury, Sc.D., W. E. Bachmann, Ph.D., and M. G. Lewisohn

(From the Departments of Obstetrics and Gynecology and of Chemistry of the University of Michigan, Ann Arbor, Mich.)

(Received for publication June 23, 1941)

Recent work on the synthesis and biological activity of carcinogenic and closely related compounds has been summarized in the reports of Fieser (18) and Cook and Kennaway (16). In the present paper we are reporting the biological activity of a number of new polycyclic hydrocarbons and derivatives which were synthesized for the first time in this laboratory by one of us (W. E. B.) and co-workers (L. H. Pence, J. M. Chemerda, and W. Cole). In addition we are reporting our experience with several well known carcinogenic hydrocarbons. We are interested in determining the carcinogenic activity of the compounds when applied in different solvents and by different modes of administration.

MATERIALS AND METHODS

The test animals were mice from an inbred albino strain that has been kept in this laboratory for several years. For external application of the compounds benzene was used most frequently as a solvent, but in a number of instances we applied acetone with considerable advantage. Very few of the solvents that have been used by other investigators are freely miscible with water. We were prompted to use acetone as a solvent on the hypothesis that a water-miscible solvent might carry the carcinogen into the cells in solution more readily. Recently Cook, Kennaway, and their associates (13, 16) mentioned the use of this solvent for the application of several compounds. Two or three drops of the solution were applied to the skin of the interscapular region twice weekly by means of a medicine dropper.

For subcutaneous injection, sesame oil and propylene glycol were used as solvents. Sesame oil is absorbed very slowly and frequently forms an oil cyst which is palpable for months. Since most of the hydrocarbons which we used are rather insoluble in propylene glycol, they were injected in the form of fine suspensions in this solvent. The propylene glycol was absorbed and the crystalline material was deposited in the subcutaneous tissue. A complication arose with certain toxic compounds which produced local ulceration with subsequent drainage through the open wound. Under these circumstances the material being tested was often lost before healing was complete with the result that no tumors were produced.

Periodic observations were made on the condition of the skin and on the size of the subcutaneous nodules of the treated mice. Papillomas, ulcerated areas and definite tumor masses were biopsied or excised and fixed in 10 per cent formalin solution. Although in many cases papillomas and tumor masses were grossly evident, only those which were diagnosed as sarcomas or carcinomas were listed as positive for induced malignancy. Since malignant neoplasms, either spontaneous or induced, rarely metastasize in mice, metastasis was not considered a necessary criterion of malignancy in this study.

Two hundred and thirty-three mice out of a total of nearly 1000 used in this investigation were represented in pathological reports on local lesions or other abnormal tissues.[1]

* This investigation was aided by a grant from the Horace H. Rackham School of Graduate Studies to Dr. N. F. Miller.

[1] All of the tissues were prepared by the Department of Pathology of this University and were diagnosed by Dr. Carl V. Weller. We wish to take this opportunity to express our appreciation to Dr. Weller for his generous assistance and continued interest in this investigation.

Derivatives of 1,2-Benzanthracene

9,10-Dimethyl-1,2-benzanthracene (I, Fig. 3) was prepared in this laboratory by a procedure applicable to the preparation of a variety of 9,10-dialkyl derivatives (4). This compound has been shown to be a potent carcinogen by Bachmann, Kennaway, and Kennaway (9), who reported the production of tumors in mice as early as 35 days after the first application of the compound. Their results show that when painted on the skin the latent period of 9,10-dimethyl-1,2-benzanthracene is about half that of 3-methylcholanthrene[2] which up to that time had been considered to be the most potent carcinogen. Of 20 mice painted with a 0.3 per cent benzene solution of the hydrocarbon 13 developed tumors (8 epitheliomas and 5 papillomas). Law and Lewisohn (23) painted a 0.3 per cent benzene solution of 9,10-dimethyl-1,2-benzanthracene on the skin of mice of several inbred strains. The animals of a subline of the dilute brown (Dba) strain died of induced lymphomatosis before any neoplasia of the skin were observed. On the other hand all the C57 brown mice developed multiple papillomas and a few developed carcinomas after 2 to 3 months' application.[3]

The rapid production of tumors by 9,10-dimethyl-1,2-benzanthracene which has been reported previously, has been confirmed by us (Table I). Fourteen mice were painted with a 0.3 per cent solution of the hydrocarbon in acetone for 6 weeks. During this time the solution was kept in a glass stoppered bottle covered with black paper to prevent photochemical oxidation to which the compound is susceptible. During the 2nd and 3rd weeks of application there was a definite loss of hair over the treated area, and the skin became thickened. Then the skin became dry and scaly and excessive keratinization was evident. Later there were ulcerated areas that healed with such extensive scar formation that in many instances it restricted the motion of the leg.

Thickened areas of the skins of 2 mice were biopsied 32 days after the initial application, and a positive diagnosis of squamous cell carcinoma was made in both cases (Fig. 1). During the 4th month 4 of the 9 surviving mice had tumors. Biopsies and autopsies of

Table I: Results of Percutaneous Application of 0.3 Per Cent Solutions of Carcinogenic Agents *

Compound	Solvent	Number of mice	Month during which positive evidence of malignancy appeared								"Average" latent period in months
			1	2	3	4	5	6	7	8 & over	
3-Methylcholanthrene	Acetone	15	15—	15—	15—	5+ 10—	9+ 6—	13+ 2—	14+ 1—	....	4.6
3-Methylcholanthrene	Benzene	15	15—	15—	15—	2+ 12—	7+ 3—	7+ 3—	8+ 2—	10+ 0—	5.2
meso-Dihydrocholanthrene	Acetone	14	0+ 14—	0+ 13—	0+ 13—	0+ 13—	0+ 13—	0+ 13—	0+ 13—	0+ 11—	0
meso-Dihydrocholanthrene	Benzene	15	0+ 15—	0+ 15—	0+ 15—	0+ 15—	0+ 15—	0+ 15—	0+ 14—	0+ 14—	0
9,10-Dimethyl-1,2-benzanthracene.	Acetone (protected from light).	14	14—	2+† 12—	4+ 7—	4+ 5—	4+ 3—	Terminated			2.0
9,10-Dimethyl-1,2-benzanthracene.	Benzene (not protected from light).	15	15—	15—	13—	2+ 12—	2+ 11—	2+ 10—	2+ 10—	3+ 8—	5.0
9,10-Dimethyl-1,2-benzanthracene.	Acetone (not protected from light).	30	29—	1+ 28—	1+ 28—	4+ 25—	4+ 21—	4+ 19—	8+ 8—	....	4.7
5-*n*-Propyl-9,10-dimethyl-1,2-benzanthracene.	Acetone	13	13—	13—	13—	2+ 9—	4+ 7—	5+ 4—	5+ 3—	0—	4.5
5-*n*-Propyl-9,10-dimethyl-1,2-benzanthracene.	Benzene	14	14—	14—	8—	1+ 4—	3+ 2—	3+ 1—	Terminated		4.3

* In this table and in Table II the + sign indicates the number of mice which have been positive up to and including the indicated month; the — sign indicates the number still living and negative in that month.

† Tumors appeared 32 days after first application (Fig. 1).

[2] The numbering system of the cholanthrene molecule is that employed in the index of *Chemical Abstracts*. On Fieser's system this compound is called 20-methylcholanthrene.

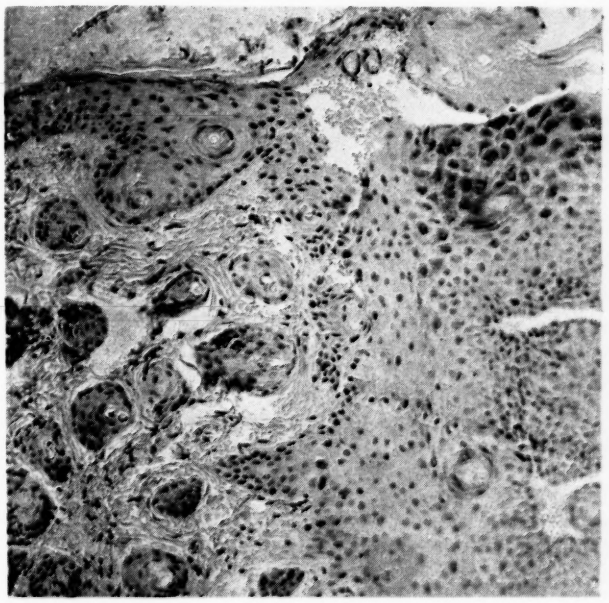
[3] Personal communication.

8 mice at later dates showed presence of malignant tissues in four. The cancerous area apparently regressed completely in one of the two mice showing carcinoma at 32 days, since no definite malignant change was found in a specimen removed in a biopsy taken 7 weeks later, and the mouse survived an additional 6 weeks without any evidence of recurrence of malignancy.

Several biopsies of the skin after 10 days of application showed complete necrosis of the epidermis. This necrosis may account for the relatively low incidence of malignancies which we obtained with this compound, in comparison with the number produced by such compounds as 3-methylcholanthrene which is less toxic to the skin (Table I). This necrosis may account also for the simultaneous occurrence of a squamous cell carcinoma superimposed upon a spindle cell sarcoma, since this condition would allow the reagent to come into contact with the underlying connective tissue. There were 3 cases in which the dermal application of 9,10-dimethyl-1,2-benzanthracene resulted in the simultaneous formation of a spindle cell sarcoma and squamous cell carcinoma.

Further investigation is desirable to determine whether more dilute solutions of 9,10-dimethyl-1,2-benzanthracene with their lowered toxicity would yield a higher number of malignancies. This possibility is suggested by the fact that a number of malignancies developed in sites remote from the area of local application. Pertinent in this connection is the report that a 0.06 per cent solution of the carcinogen 5,9,10-trimethyl-1,2-benzanthracene was more effective than a 0.3 per cent solution in producing epitheliomas in mice (16). We have found in agreement with others that the toxicity of some of these compounds and the prolonged irritation of benzene sometimes leads to systemic conditions which may kill the mouse before it has a chance to show neoplastic changes at the site of application. It was also noted that papillomas sometimes regressed with continued treatment. It is apparent that toxicity is one of the major factors affecting the so-called potency and latent period of a carcinogenic agent.

We wish to emphasize that solutions of 9,10-dimethyl-1,2-benzanthracene must be kept tightly stoppered and protected from light in order to prevent photochemical oxidation of the hydrocarbon to the inactive photo-oxide. A benzene solution of the hydrocarbon kept on an open shelf unprotected from light gradually loses its potency. In one experiment 15 mice were painted twice weekly for 10 weeks with such an exposed solution. Only 3 mice developed malignant tumors at the site of application, and after 1 year 8 mice still survived, but all of these had adenomas of the lung. These results are in harmony with those reported by Cook and Martin (17) who have isolated



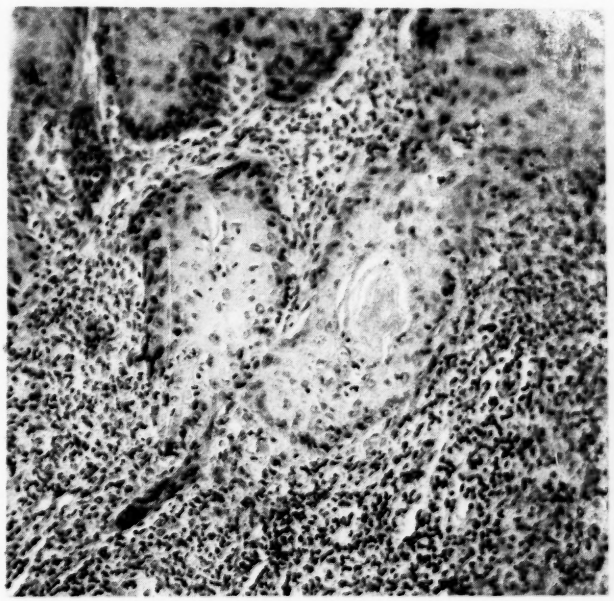
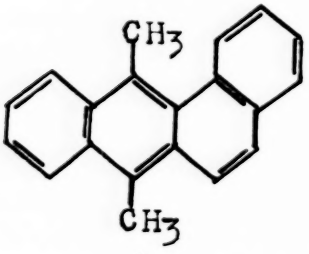
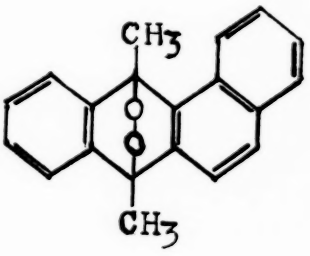


FIG. 1.—Photomicrograph of section of early cornifying squamous cell carcinoma in a mouse. This mouse had been treated with 0.3 per cent solution of 9,10-dimethyl-1,2-benzanthracene in acetone twice weekly during the 32 days preceding the biopsy at which this specimen was taken. Mag. × 180.

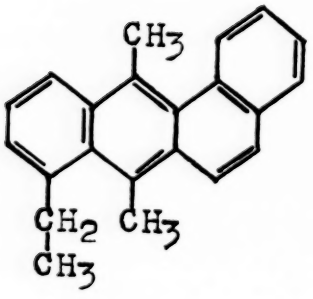
FIG. 2.—Photomicrograph of section of squamous cell carcinoma and spindle cell fibrosarcoma in a mouse. This animal had been treated with a 0.3 per cent solution of 3-methylcholanthrene in benzene twice a week for 9 weeks. A biopsy taken after 4.5 months was diagnosed as early cornifying squamous cell carcinoma. This tissue, taken at autopsy two months later, shows the presence of a second neoplastic process. Mag. × 180.



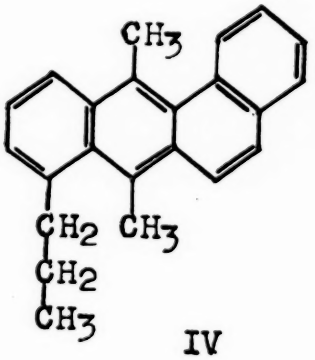
I



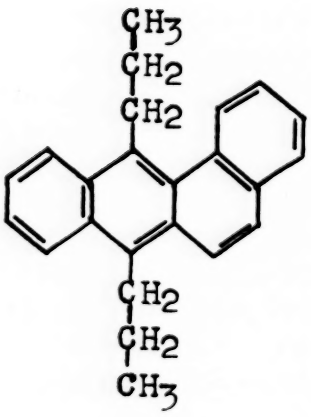
II



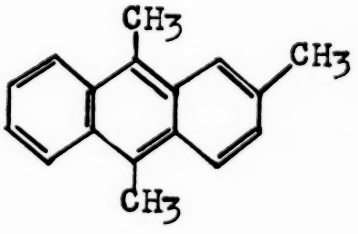
III



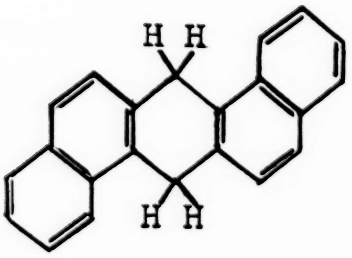
IV



V



VI



VII

FIG. 3.—Structural formulas of compounds I to VII.

the photo-oxide formed from 9,10-dimethyl-1,2-benzanthracene and have shown it to have the structure II (Fig. 3). They found that tumors did not result when a suspension of the pure photo-oxide in sesame oil was injected into mice.

In agreement with previous observation (9, 24), 9, 10-dimethyl-1,2-benzanthracene was found to be very toxic when administered subcutaneously. A dose of 5 mgm. of the hydrocarbon in 0.1 cc. of sesame oil produced a marked generalized edema within a few hours in the 9 mice that were injected. Six of these mice died in 3 weeks. One of the two mice that survived for 4 months developed a spindle cell fibrosarcoma; the other developed a large tumor which was not examined microscopically.

A suspension of 5 mgm. of 9,10-dimethyl-1,2-benzanthracene in 0.2 cc. of propylene glycol was injected into 9 mice. Four of these died within 24 hours; of the 4 mice which survived 3 months, 2 had tumors, one a spindle cell sarcoma and the other a squamous cell carcinoma with metastasis to the submaxillary lymph nodes. In this case some of the material may have been injected intradermally or leaked back along the needle puncture to give rise to a carcinoma. Bachmann, Kennaway, and Kennaway (9) recorded the formation of an epithelioma induced by subcutaneous injection of this hydrocarbon. In our injection experiments, the few animals which survived the initial toxicity showed a higher incidence of induced malignancy (4 out of 6) than was observed in the painting experiments. The average latent period was, however, longer than in the painting experiments, being 3 to 4 months.

New Derivatives of Benzanthracene

Preliminary observations have been made on two new hydrocarbons, 5-ethyl-9,10-dimethyl-1,2-benzanthracene (III, Fig. 3) (5) and 5-*n*-propyl-9,10-dimethyl-1,2-benzanthracene (IV, Fig. 3). A 0.3 per cent solution of the 5-ethyl-compound in benzene was applied to the skin of 10 mice for 10 weeks. It proved to be so toxic that all the mice died before the 6th month and none had developed lesions suggestive of a malignant change (Table III).

Table II: Results of Subcutaneous Injections of Carcinogens in Sesame Oil

Compound	Dose	Number of mice	Month during which positive evidence of malignancy appeared								"Average" latent period in months
			1	2	3	4	5	6	7	8 & over	
1,2,5,6-Dibenzanthracene	3 mgm.	12	12—	11—	11—	3+ 8—	4+ 7—	6+ 5—	7+ 4—	....	4.8
Cholanthrene	5 mgm.	13	12—	12—	11+ 1—	11+ 1—	Terminated				2.5
3-Methylcholanthrene	5 mgm.	14	14—	5+ 9—	8+ 6—	11+ 3—	12+ 1—	12+ 1—	12+ 1—	13+ 0—	2.9
4-Methylcholanthrene	2 mgm.	11	11—	3+ 5—	3+ 5—	6+ 0—	....	....	....	....	2.4
5-Methylcholanthrene	2 mgm.	16	16—	16—	16—	3+ 13—	6+ 6—	7+ 4—	Terminated		4.2
meso-Dihydrocholanthrene	2.5 mgm.	29	29—	2+ 27—	6+ 23—	15+ 14—	18+ 11—	21+ 5—	21+ 5—	....	3.6
meso-Dihydro-3-methylcholanthrene.	5 mgm.	10	10—	10—	1+ 8—	3+ 3—	4+ 1—	4+ 0—	....	....	3.7
Ethyl ester of 1:2:5:6-dibenzanthracene-*endo*-α,β-succino glycine.	5 mgm.	13	13—	13—	13—	9—	1+ 8—	1+ 8—	1+ 8—	3+ 6—	6.4
Ethyl ester of 3-methylcholanthrene-*endo*-α,β-succino glycine.	5 mgm.	16	16—	16—	4+ 7—	5+ 6—	7+ 1—	7+ 1—	8+ 0—	....	3.6

The 5-*n*-propyl- derivative in a 0.3 per cent solution in acetone and also in benzene was applied to two groups of mice for 10 weeks. Of 13 mice painted with the acetone solution, 4 had developed tumors by the 5th month, while of the 14 mice treated with the benzene solution only 3 had developed carcinomas by the 6th month (Table I). Subcutaneously 5-*n*-propyl-9,10-dimethyl-1,2-benzanthracene is somewhat less active than 9,10-dimethyl-1,2-benzanthracene. Of 11 mice given a single dose of 5 mgm. of the hydrocarbon in sesame oil, 4 developed sarcomas during the 9 months of observation. Of 8 mice which received 5 mgm. in

propylene glycol, 2 developed sarcomas during the sixth month (Table III).

The replacement of the two methyl groups in the 9,10-positions by two *n*-propyl groups destroyed or greatly reduced the toxic and carcinogenic properties of the molecule. A suspension of 3 mgm. of 9,10-di-*n*-propyl-1,2-benzanthracene (V, Fig. 3) in 0.2 cc. of propylene glycol injected into mice was not noticeably toxic; and after 10.5 months all the mice were living and exhibited no evidence of neoplasia. We also tested 2,9,10-trimethyl-anthracene (VI, Fig. 3) (6) which corresponds to 9,10-dimethyl-1,2-benzanthracene with the benzo group replaced by one methyl group. Injection of 2 mgm. of this compound in sesame oil failed to produce tumors in 15 mice during an eight month period of observation (Table III).

9,10-Dihydro-1,2,5,6-dibenzanthracene (VII, Fig. 3) was prepared in pure form from the carcinogen 1,2,5,6-dibenzanthracene by a procedure employed in this laboratory for the preparation of meso-dihydro derivatives of a number of polycyclic hydrocarbons including cholanthrene and 3-methylcholanthrene (2, 3). Ten mice were given 5 mgm. of this compound subcutaneously in sesame oil; of 7 mice that survived 9 months, only one developed a malignant tumor, a spindle cell sarcoma. Nine mice survived for 7 months and all were negative (Table III). For comparison the parent hydrocarbon 1,2,5,6-dibenzanthracene (3 mgm. in sesame oil) was injected into 12 mice; 3 of these developed tumors during the 4th month and by the 7th month 7 were positive and 4 negative (Table II).

Table III: Results of Preliminary Observations on Certain Carcinogenic Agents and Their Derivatives

Compound	Solvent and dosage *	Number of mice treated	Number positive	Number negative	Time in months
5-Ethyl-9,10-dimethyl-1,2-benzanthracene	2 mgm. in sesame oil	10	0	10	6
5-*n*-Propyl-9,10-dimethyl-1,2-benzanthracene	5 mgm. in sesame oil	11	4	7	9
5-*n*-Propyl-9,10-dimethyl-1,2-benzanthracene	5 mgm. in propylene glycol	8	2	6	6
2,9,10-Trimethylanthracene	2 mgm. in sesame oil	15	0	15	8
9,10-Di-*n*-propyl-1,2-benzanthracene	3 mgm. in propylene glycol	10	0	10	10½
9,10-Dihydro-1,2,5,6-dibenzanthracene	Acetone (dermal)	16	0	9	7
9,10-Dihydro-1,2,5,6-dibenzanthracene	5 mgm. in sesame oil	10	1	6	7
1-Ketocholanthrene	5 mgm. in propylene glycol	11	0	6	5
5-Methyl-7,8-dihydro-1,2-benzanthracene	5 mgm. in sesame oil	10	0	10	7

* All compounds in sesame oil and propylene glycol were administered subcutaneously, the amount indicated being dissolved in 0.2 cc. of the solvent.

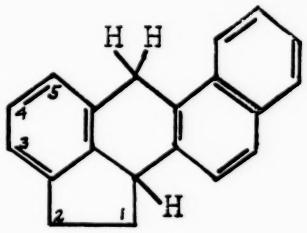
Derivatives of Cholanthrene

Two sets of 15 mice each were painted with a 0.3 per cent solution of *meso*-dihydrocholanthrene (VIII, Fig. 4) (3). For one set an acetone solution was used; for the other a benzene solution. The solutions were applied to the skin for 13 weeks in the manner described. After about three weeks of painting with the acetone solution, a thinning of the hair was observed over the treated area, but there was no true epilation or thickening of the skin, whereas with the benzene solution both epilation and thickening resulted, an indication of the greater toxicity of benzene. In the latter group the hair grew back, and no further thickening of the skin occurred after the painting was stopped. No tumors appeared in either group during the 8 months of observation (Table I). From these results it is apparent that the addition of two hydrogen atoms to the *meso*-positions of the cholanthrene molecule destroyed or greatly reduced the carcinogenic activity when applied to the skin.

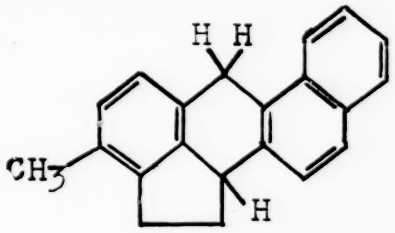
Strikingly different results were observed when the compound was injected subcutaneously. Of 29 mice which received a single injection of 2.5 mgm. of *meso*-dihydrocholanthrene in 0.2 cc. of sesame oil, 21 developed spindle cell sarcomas in an average latent period of 3.6 months; the first two tumors appearing during the 2nd month. After 8 months 5 of the animals were living and negative (Table II).

For comparison 5 mgm. of cholanthrene in 0.2 cc. of sesame oil was injected into 12 mice. Eleven developed tumors in the 13th and 14th weeks after injection (Table II).

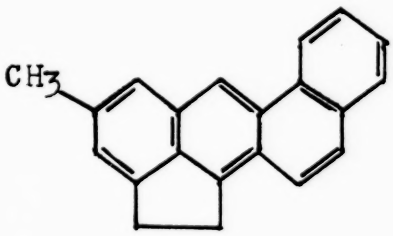
The absence of tumor formation when *meso*-dihydrocholanthrene is applied to the skin in marked contrast to the high incidence and relatively short latent period (comparable to the latent period of cholanthrene) for their production when the compound is injected, suggests the possibility that the dihydro derivative is oxidized *in vivo* by the subcutaneous connective tissue (but not by the skin) to the potent carcinogen, cholanthrene. Such a possibility has been anticipated by Cook and Kennaway (15). The possible importance of such a transformation has been discussed



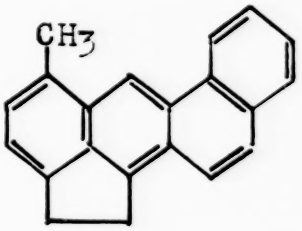
VIII



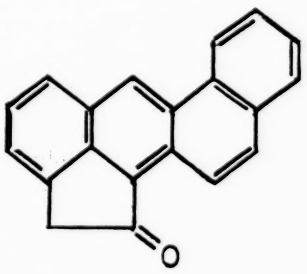
IX



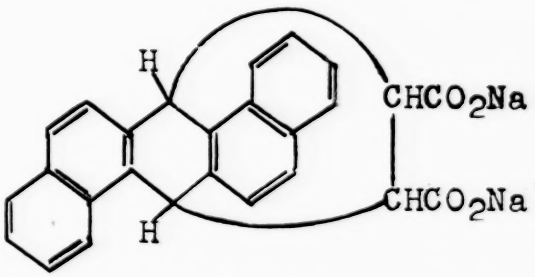
X



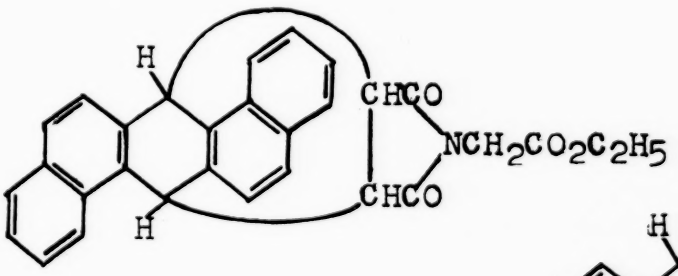
XI



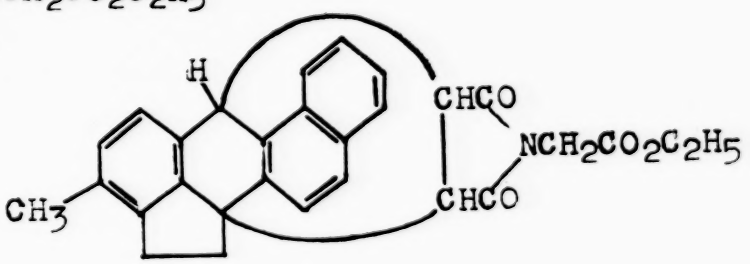
XII



XIII



XIV



XV

FIG. 4.—Structural formulas of compounds VIII to XV.

in connection with the possible formation of simple hydro derivatives of methylcholanthrene as intermediate in normal biological processes (18). Similar results with painting and injection experiments have been recorded for 1′,2′-dihydro-4′-methyl-3,4-benzpyrene, a compound which is readily converted to the corresponding aromatic hydrocarbon (18).

A group of 10 mice was injected subcutaneously with 5 mgm. of *meso*-dihydro-3-methylcholanthrene (IX, Fig. 4) (2) in 0.25 cc. of sesame oil. Four mice had developed sarcomas by the 5th month, and 4 died at this time without tumors (Table II). For comparison 5 mgm. of 3-methylcholanthrene in 0.2 cc. of sesame oil was injected into 14 mice. Thirteen of the animals developed spindle cell sarcomas over a period of 8 months; the first 5 appeared during the second month, and 12 had appeared by the 5th month. The average latent period was approximately the same as that for cholanthrene, but the range in latent period was somewhat greater for this methyl derivative (Table II).

Effects of Solvents

In order to determine the effect of the solvent in producing tumors by dermal application of 3-methylcholanthrene, two sets of 15 mice each were painted with a 0.3 per cent solution of the hydrocarbon twice weekly for 11 weeks. One set was painted with an acetone solution and the other with a benzene solution. After 4.5 months biopsies were performed on skin areas that looked grossly atypical. Of 6 biopsies on the acetone group, 5 were definitely squamous cell carcinomas and the other was diagnosed as an "atypical hyperplasia that was probably a squamous cell carcinoma." Of 4 biopsies on the benzene group, 2 were squamous cell carcinomas; one was an "atypical hyperplasia of squamous epithelium, probably an early carcinoma," and the other was negative.

After 5 months, 8 of the acetone treated group had large squamous cell carcinomas and were sacrificed. Five more that were sacrificed at 6.5 months also had carcinomas. The remaining 2 mice survived for 8 months; one of these developed a carcinoma, but the other had only a horny wart with no malignant change (Table I).

The group painted with the benzene solution did not develop malignancies comparable to those of the acetone group until 6 to 6.5 months when 6 mice were sacrificed with large squamous cell carcinomas. One of these tumors showed the presence of a spindle cell fibrosarcoma as well as the carcinoma (Fig. 2), a condition which was discussed in relation to the carcinogenic action of 9,10-dimethyl-1,2-benzanthracene.

In these experiments acetone possessed certain advantages over benzene. The general physical condition of the mice of the group treated with acetone was better than those of the group treated with benzene and the induction of malignancy was more rapid and more consistent when acetone was used as a solvent. In our experiments the latent period of tumor production by 3-methylcholanthrene was about two months longer when administered on the skin than when injected subcutaneously (Tables I and II).

New Isomers of 3-Methylcholanthrene

Three new isomers of 3-methylcholanthrene which differ only in the position of the methyl group have been synthesized recently in this laboratory. 4-Methylcholanthrene (X, Fig. 4) (19) and 5-methylcholanthrene (XI, Fig. 4) have been synthesized by Bachmann and Chemerda (7), and 7-methylcholanthrene has been prepared by Bachmann and Safir (12). We are able to report some preliminary observations on the carcinogenic activity of the first two isomers, in addition to the data presented by Law and Lewisohn (24).

Eleven mice were injected with 2 mgm. of 4 methylcholanthrene in 0.2 cc. of sesame oil. The compound proved to be so strong a local irritant that the skin sloughed away over the injection site and a large open wound was present in nearly all the mice. Of 5 mice killed after 2 months, 3 had squamous cell carcinomas and the others were negative. The carcinomas may have resulted from the carcinogen draining from the wound onto the skin. After 4 months, 4 of the remaining 6 mice had developed neoplasmas (Table II).

Twelve mice were injected with 2 mgm. of 5-methylcholanthrene in 0.2 cc. of sesame oil. By the 4th month, 3 had developed large malignant tumors; 3 more were found with sarcomas during the 5th month, and 4 mice were surviving and negative when the experiment was terminated at 6 months (Table II).

The introduction of an oxygen atom for two hydrogen atoms on the 1-carbon atom of the cholanthrene molecule destroyed its carcinogenic activity, for no tumors developed when a suspension of 1-ketocholanthrene (XII, Fig. 4) (3) in propylene glycol was injected into 11 mice, although 6 of the mice lived for 7 months after the injection (Table III).

Carcinogen-glycine Compounds

A procedure has been described for preparing in excellent yields the maleic anhydride addition products of a number of carcinogens from which water-soluble salts can be prepared (3, 10). An example of this type of compound is shown in XIII (Fig. 4) which is the formula for the disodium salt of 1,2,5,6-dibenzanthracene-9,10-*endo*-α,β-succinic acid. Sarcomas have been produced by successive subcutaneous injections of an aqueous solution of this compound

(14, 16, 20). Generally the tumors were produced by repeated injections which in some cases were continued until the tumors developed (4.5 to 6 months). With the sodium salt of 3-methylcholanthrene-*meso-endo-α,β*-succinic acid, sarcomas were produced in about half this time (16). We have combined the addition products obtained from 1,2,5,6-dibenzanthracene and 3-methylcholanthrene with the ethyl ester of glycine, and obtained the products shown in XIV and XV (Fig. 4) (8). As shown in Table II both compounds proved to be active carcinogens.

Thirteen mice received 5 mgm. of the adduct of 1,2,5,6-dibenzanthracene *meso-endo-α,β*-succinic acid and the ethyl ester of glycine (XIV, Fig. 4) in 0.2 cc. of sesame oil. The first tumor appeared during the 5th month and two more tumors appeared in the 9th month when 6 other mice were alive and negative and the experiment was terminated. It is seen that the latent period of this adduct is comparable to that of its parent hydrocarbon 1,2,5,6-dibenzanthracene (Table II).

In a similar experiment with the 3-methylcholanthrene adduct (XV, Fig. 4), 16 mice were given a subcutaneous injection of 5 mgm. of this compound in 0.2 cc. of sesame oil. Four malignancies appeared during the 3rd month, and by the 6th month 7 of the remaining 9 mice were positive for induced malignancy. The latent period of this carcinogen is also roughly the same as that for its parent hydrocarbon, 3-methylcholanthrene. (Table II). Neither of the ethyl ester of glycine adduct products was toxic, and all the tumors obtained were spindle cell fibrosarcomas.

Lung Tumors

During the course of this investigation it became apparent that there was a high incidence of adenomas and adenocarcinomas in the lungs of treated animals that survived 8 months or longer, even though no neoplasm developed at the site of application. The incidence of lung tumors was practically nil in several groups of untreated control mice of the same age. Andervont (1) has discussed the increased incidence of lung tumors in mice under various conditions of treatment with carcinogenic compounds. It is clear from his work that there are many factors involved in the induction of lung tumors, and that carcinogenic agents may cause a marked increase in the incidence of such tumors.

Summary

1. 9,10-Dimethyl-1,2-benzanthracene was found to produce carcinoma as early as 32 days after the first dermal application, but its toxicity was so great that the percentage of induced malignant tumors was not large. Because of susceptibility of the compound to photo-oxidation, solutions of this carcinogen should be well protected from light.

2. *Meso*-dihydrocholanthrene was found to have an average latent period of 3.6 months when injected subcutaneously. Solutions painted on the skin did not give rise to neoplastic changes, but did cause adenomas of the lungs. Two other *meso*-dihydro compounds were found to be carcinogenic when applied subcutaneously.

3. Other new chemical compounds which proved to be carcinogenic agents are: 5-*n*-propyl-9,10-dimethyl-1,2-benzanthracene, 4-methylcholanthrene and 5-methylcholanthrene. Two carcinogen-glycine compounds, namely the ethyl esters of 3-methylcholanthrene-*meso-α,β-endo*-succinoyl-glycine, and 1,2,5,6-dibenzanthracene-*meso-α,β-endo*-succinoyl-glycine also proved to be carcinogenic agents with latent periods approximately the same as their respective parent hydrocarbons.

4. A water-miscible solvent, acetone, was found to have a definite accelerating effect on the action of certain carcinogenic agents when applied to the skin.

5. The toxicity of a substance may cause so much local tissue necrosis that its carcinogenic effect may be masked. Lung tumors or other systemic conditions may lead to the death of an animal before the local induction of carcinoma or sarcoma has had time to develop.

References

1. Andervont, H. B. Pulmonary Tumors in Mice. I. The Susceptibility of the Lungs of Albino Mice to the Carcinogenic Action of 1:2:5:6-Dibenzanthracene. Pub. Health Rep., **52**:212-221. 1937.

2. Bachmann, W. E. The Reaction of Alkali Metals with Polycyclic Hydrocarbons: 1,2-Benzanthracene, 1:2:5:6-Dibenzanthracene and Methylcholanthrene. J. Org. Chem., **1**: 347-353. 1936.

3. Bachmann, W. E. The Synthesis of Compounds Related to 1,2-Benzanthracene and Cholanthrene. J. Org. Chem., **3**:434-437. 1938.

4. Bachmann, W. E., and J. M. Chemerda. Synthesis of 9,10-Dimethyl-1,2-benzanthracene, 9,10-Diethyl-1,2-benzanthracene, and 5,9,10-Trimethyl-1,2-benzanthracene. J. Am. Chem. Soc., **60**:1023-1026. 1938.

5. Bachmann, W. E., and J. M. Chemerda. The Synthesis of 9,10-di-Alkyl-1,2-benzanthracenes. J. Am. Chem. Soc., **61**:2358-2361. 1939.

6. Bachmann, W. E., and J. M. Chemerda. The Synthesis of 9,10-di-Alkylanthracenes. J. Org. Chem., **4**:583-587. 1939.

7. Bachmann, W. E., and J. M. Chemerda. The Synthesis of 4-Methylcholanthrene and 5-Methylcholanthrene. J. Org. Chem., **6**:50-53. 1941. (See also 19.)

8. Bachmann, W. E., and W. Cole. The Coupling of Derivatives of Polycyclic Hydrocarbons with Glycine. J. Org. Chem., **4**:60-67. 1939.

9. Bachmann, W. E., E. L. Kennaway, and N. M. Kennaway. The Rapid Production of Tumors by Two New Hydrocarbons. Yale J. Biol. Med., **11**:97-102. 1938.
10. Bachmann, W. E., and M. C. Kloetzel. Reactions Between Maleic Anhydride and Polycyclic Hydrocarbons. J. Am. Chem. Soc., **60**:481-485. 1938.
11. Bachmann, W. E., and L. H. Pence. Reaction of Alkali Metals with Polycyclic Hydrocarbons. II. J. Am. Chem. Soc., **59**:2339-2342. 1937.
12. Bachmann, W. E., and S. R. Safir. 7-Methylcholanthrene and 1′,5-Dimethyl-1,2-benzanthracene. J. Am. Chem. Soc., **63**:855-857. 1941.
13. Badger, G. M., J. W. Cook, C. L. Hewett, E. L. Kennaway, N. M. Kennaway, R. H. Martin, and A. M. Robinson. The Production of Cancer by Pure Hydrocarbons. V. Proc. Roy. Soc. London, Ser. B., **129**:439-467. 1940.
14. Barry, G., J. W. Cook, G. A. D. Haslewood, C. L. Hewett, I. Hieger, and E. L. Kennaway. The Production of Cancer by Pure Hydrocarbons. Part III. Proc. Roy. Soc. London, Ser. B., **117**:318-351. 1935.
15. Cook, J. W., and E. L. Kennaway. Chemical Compounds as Carcinogenic Agents. First Supplementary Report: Literature of 1937. Am. J. Cancer, **33**:50-97. 1938.
16. Cook, J. W., and E. L. Kennaway. Chemical Compounds as Carcinogenic Agents. Second Supplementary Report: Literature of 1938 and 1939. Am. J. of Cancer, **39**:381-428. 1940.
17. Cook, J. W., and R. H. Martin. Polycyclic Aromatic Hydrocarbons. Part XXIV. J. Chem. Soc., **2**:1125-1127. 1940.
18. Fieser, L. F. Carcinogenic Activity, Structure and Chemical Reactivity of Polynuclear Aromatic Hydrocarbons. Am. J. Cancer, **34**:37-124. 1938.
19. Fieser, L. F., and D. M. Bowen. New Methyl and Dimethyl Derivatives of Cholanthrene. J. Am. Chem. Soc., **62**:2103-2108. 1940.
20. Haddow, A. The Influence of Carcinogenic Substances on Sarcomata Induced by the Same and Other Compounds. J. Path. and Bact., **47**:581-591. 1938.
21. Haddow, A., and A. M. Robinson. The Influence of Various Polycyclic Hydrocarbons on the Growth Rate of Transplantable Tumors. Proc. Roy. Soc. London, Ser. B., **122**:442-446. 1937.
22. Hieger, I. On the Mechanism of Carcinogenesis by Chemical Compounds. Am. J. Cancer, **28**:522-529. 1936.
23. Law, L. W., and M. G. Lewisohn. Induction of Lymphamatosis in Mice Following Painting with 9,10-Dimethyl-1,2-benzanthracene. Proc. Soc. Exper. Biol. & Med., **43**:143-146. 1940.
24. Law, L. W., and M. Lewisohn. Comparative Carcinogenicity of Some Cholanthrene Derivatives. Cancer Research, **1**:695-698. 1941.
25. Shear, M. J. Studies in Carcinogenesis. V. Methyl Derivatives of 1,2-Benzanthracene. Am. J. Cancer, **33**:499-537. 1938.

Comparative Carcinogenicity of Some Cholanthrene Derivatives*

L. W. Law, Ph.D.,† and Marjorie Lewisohn‡

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

(Received for publication June 13, 1941)

Biological testing of the hydrocarbons, cholanthrene and methylcholanthrene, has shown these two compounds to be carcinogenic agents of great potency. Because of the relationship of the cholanthrene group to the cholane class (bile acids) of naturally occurring substances, considerable interest and importance have been attached to these compounds. Methylcholanthrene was first prepared by the London (3) and Harvard (5) groups from the naturally occurring bile acids, desoxycholic and cholic acid, respectively.

Numerous investigations of the carcinogenicity of compounds related to cholanthrene, the parent hydrocarbon, have been reported (4, 6, 7, 8). Although none of the compounds tested has shown a carcinogenic potency as great or greater than that of cholanthrene or methylcholanthrene, the results indicate certain influences of modification of molecular structure on carcinogenic activity. The following conclusions have been drawn from the results of biological testing: The parent hydrocarbon, cholanthrene, does not differ appreciably from methylcholanthrene in behavior noted in either subcutaneous injection or painting experiments. Thus it is concluded that the methyl group of 3-methylcholanthrene does not contribute significantly to its carcinogenic activity. Various elaborations of the cholanthrene molecule, by substitutions and additions, beyond the introduction of a single methyl group at position 3 (20 in the sterol-bile acid numbering system) result in a considerable decrease in carcinogenic activity. On the other hand, simplification of the cholanthrene molecule has resulted in the recognition that carcinogenic potency of a high order is associated with simple meso-alkyl 1,2-benzanthracenes, particularly 10-methyl-1,2-benzanthracene.

The present report concerns a comparison of the carcinogenic properties of cholanthrene and five of its derivatives: 3 isomeric methylcholanthrenes and dihydrocholanthrenes. The methyl derivatives have their methyl groups at positions 3, 4, and 5, respectively. The synthesis of these compounds was described by Bachmann and Chemerda (2). The two dihydro derivatives used were *meso*-dihydro-cholanthrene and *meso*-dihydro-3-methylcholanthrene. These compounds were prepared by a reaction of the hydrocarbon with sodium followed by alcoholysis of the alkali derivative as described by Bachmann (1).

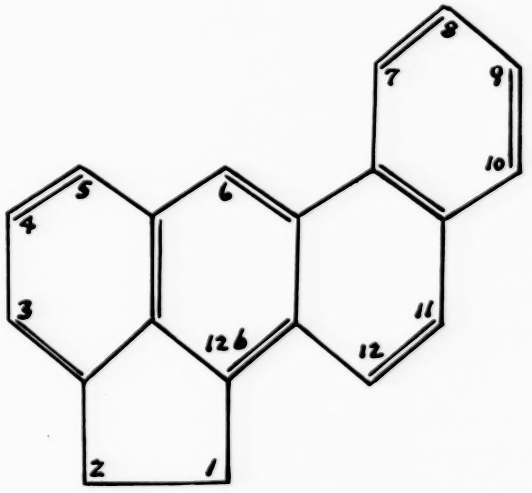


FIG. 1.—Numbering system used for cholanthrene molecule.

MATERIALS AND METHODS

Carcinogenicity of the cholanthrene derivatives was determined by I. the incidence and latent period of induced subcutaneous growths, and II. the incidence and extent of reaction of primary carcinomas of the lung.

Inbred Bragg-MacDowell C strain albino mice were used as test animals. This strain has been maintained by brother to sister matings for over 45 generations. The strain was obtained by Dr. G. D. Snell of this Laboratory from Dr. E. C. MacDowell, at the 25th generation of inbreeding. There is a relatively low incidence of spontaneous tumors of connective tissue and breast, but a relatively high incidence of pulmonary carcinomas (±50 per cent) late in life. Of 33 control C strain mice inoculated with olive oil and

* Grateful acknowledgment is made to Dr. W. E. Bachmann, Department of Chemistry, University of Michigan, who supplied the cholanthrene derivatives.

† Finney-Howell Foundation Fellow.

‡ Summer student from The Johns Hopkins University School of Medicine.

necropsied at 7 months, only one had a primary lung carcinoma while among 39 uninjected mice killed at the same age there was found only a single lung tumor.

Equal numbers of males and females were inoculated in the right axilla at 1 month of age with 1 mgm. of the carcinogen dissolved in 0.25 cc. of a purified commercial olive oil. Mice developing subcutaneous growths were necropsied approximately two weeks following the appearance of a palpable nodule. Mice which failed to develop subcutaneous growths were killed at 7 months of age.

Results

There appeared sarcomas in 90 per cent of the mice receiving the parent hydrocarbon, cholanthrene. The mean latent period was 11.7 ± 0.69 weeks. Addition of a methyl group at position 3 (3-methylcholanthrene) or at position 4 (4-methylcholanthrene) did not significantly alter the incidence of induced sarcomas or their time of appearance. However, substitution of a methyl group at the 5 position (5-methylcholanthrene) in the cholanthrene molecule resulted in a decided decrease in incidence of sarcomas (71.4 per cent) and a significant lengthening of the latent period (15.8 ± 0.62 weeks) as compared with the reactions to cholanthrene under similar conditions (Table I).

Hydrogenation of either the cholanthrene or 3-methylcholanthrene molecule did not significantly change the response in sarcoma production of the test animals.

The response of lung tissue in the formation of neoplasms was much at variance with the reaction of connective tissue. 3-Methylcholanthrene was more potent than the parent hydrocarbon in inducing lung carcinoma. Statistical significance in all cases was tested by the method of unpaired comparisons by calculating the standard error from combined data.[1] The difference between the per cent induction of lung carcinomas in these two groups was 43.6 ± 14.2 ($p = 0.002$). 4-Methylcholanthrene proved to be the most potent of the isomers, both in regard to percentage induction of lung carcinomas and extent of response as measured by the total number of lung nodules present at necropsy. The difference of the induced lung carcinoma ratios between this carcinogen and cholanthrene was 57.7 ± 13.3 per cent ($p = 0.0001$). In this series the majority of individuals showed extreme involvement of lung tissue in neoplasia (Table II).

The 5-methyl isomer on the other hand was only very slightly carcinogenic, inducing only 6.9 per cent lung carcinomas. This figure is not significantly different from the incidence of lung carcinoma in control mice necropsied at 7 months. Thus substitution of a methyl group at the 5 position in the cholanthrene molecule significantly reduced the carcinogenicity of the molecule. The difference of ratios between the cholanthrene and 5-methylcholanthrene groups was 27.7 ± 10.8 per cent ($p = 0.014$). Whereas the two dihydro derivatives used in this study did not differ from the parent molecules in inducing subcutaneous tumors, they were considerably less potent in inducing lung carcinomas. Although the difference of ratios of induced carcinomas is significant only as regards the 3-methylcholanthrene as compared with its dihydro derivative ($p = 0.0001$) both are in the same direction.

[1] Standard error of difference of ratios $= \sqrt{pq\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}$

Discussion

These results are of interest because they indicate that a change in the molecule of a carcinogen may affect the neoplastic response of one tissue but not that of another. Substitution of a methyl group at positions 3 or 4 in the cholanthrene molecule or hydrogenation of the cholanthrene or 3-methylcholanthrene molecule did not significantly alter the carcinogenic properties of these compounds as determined by the induction of subcutaneous tumors. On the other hand both the 3 and 4 methyl derivatives were significantly more potent as carcinogens than the parent hydrocarbon, cholanthrene, and there resulted a decrease in carcinogenicity following hydrogenation when carcinogenesis was measured by the neoplastic response of the lung tissue.

These results also show that the addition of a methyl group to the cholanthrene molecule may affect to a great extent its carcinogenic properties in one direction or the other. Although the only difference in carcinogenic power noted among the compounds tested as regards the induction of sarcomas was the decrease in carcinogenicity following substitution of a methyl group at the 5 position, substitution of methyl groups at positions 3 and 4 significantly increased the induction of lung tumors while the presence of a methyl group at position 5 decreased the power of carcinogenesis. This decrease is of statistical significance when the incidence of lung carcinomas induced by the methyl isomer is compared with either the incidence obtained by the parent hydrocarbon or by either the 3 or 4 methyl isomer.

Summary and Conclusions

1. A comparison of the carcinogenic action of cholanthrene and 5 of its derivatives, 3 isomeric methylcholanthrenes and 2 different dihydrocholanthrenes has been investigated.

2. There was found no difference in the potencies of 3 or 4 methylcholanthrene as compared with cholan-

TABLE I: PRODUCTION OF SARCOMAS IN C STRAIN MICE FOLLOWING SUBCUTANEOUS INJECTIONS OF SOME CHOLANTHRENE DERIVATIVES

Compound	No. of mice, effective total	No. sarcoma	Per cent sarcoma	Appearance in weeks following injection																Mean latent period	Coefficient of variation
				7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
Cholanthrene	30	27	90.0	1	2	6	7	1	1	2	1	2	3	..	..	..	..	..	..	1	11.7 ± 0.69	30
3-Methylcholanthrene	28	25	89.3	..	1	10	4	4	2	2	..	2	..	..	..	..	..	..	..	..	10.4 ± 0.37	18.5
4-Methylcholanthrene	29	26	89.5	..	..	1	..	7	1	9	2	..	2	2	1	1	..	..	..	..	13.3 ± 0.49	26.1
5-Methylcholanthrene	28	20	71.4	..	..	..	2	..	..	2	4	..	6	..	3	1	..	1	..	1	15.8 ± 0.62	20.8
meso-Dihydrocholanthrene	28	26	92.8	..	..	..	5	4	2	4	2	4	5	..	..	..	..	..	..	..	13.0 ± 0.42	16.9
meso-Dihydro-3-methylcholanthrene	28	23	82.2	..	..	3	7	6	..	1	..	4	2	..	..	..	..	..	..	..	11.9 ± 0.45	19.8

TABLE II: RESPONSE OF LUNGS OF C STRAIN MICE TO SUBCUTANEOUS INJECTIONS OF SOME CHOLANTHRENE DERIVATIVES

Compound	Age in weeks and reaction type of lungs at necropsy															No. necropsied	No. positive	Per cent positive	Mean no. nodules	Summation of reaction types	
	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26						
Cholanthrene	3–0*	2–1 7–0	...	3–1	1–0	2–0	1–0	1–1 3–0	...	1–1	...	...	...	...	2–1	26	9	34.6	3.0	17–0 9–1	...
3-Methylcholanthrene	6–1	6–1 2–0	1–1 1–0	4–1	...	...	1–0	...	1–2	...	...	...	...	...	1–0	23	18	78.3	2.5	5–0 17–1	1–2
4-Methylcholanthrene	...	1–3 4–1 1–0	1–3 1–1	2–4 3–2 2–1	1–4	1–3	3–4 1–3	1–4	1–4 1–3 1–1	...	...	...	...	...	1–0	26	24	92.3	14.8	2–0 8–1 3–2	5–3 8–4
5-Methylcholanthrene	...	1–0	...	2–0	1–0	2–0	...	1–1 5–0	3–0	1–1 2–0	1–0	...	...	...	10–0	29	2	6.9	1.0	27–0 2–1	...
meso-Dihydrocholanthrene	3–0	..	...	1–1 4–0	6–0	2–1 1–0	1–1 3–0	2–0	1–4	1–0	...	...	...	...	1–0	26	5	19.2	5.4	21–0 4–1	1–4
meso-Dihydro-3-methylcholanthrene	...	3–1 9–0	1–0	2–0	1–1	1–0	...	4–0	1–0	1–0	...	...	...	...	3–0	26	4	15.4	1.5	22–0 4–1	...

* Number at left in each age period represents number of mice. Number at right represents type of reaction of lungs to carcinogen. 0=no tumor; 1=positive, 1–5 lung nodules; 2=5–10 nodules; 3=10–20 nodules; 4=20 or more nodules.

threne as regards induction of subcutaneous tumors (sarcomas). 5-Methylcholanthrene, however, was a less potent compound both as regards incidence of induced tumors and length of latent period. The two dihydro derivatives tested did not differ significantly from the parent compounds.

3. Both 3-methylcholanthrene and 4-methylcholanthrene were significantly more potent in inducing lung carcinomas than the parent hydrocarbon, cholanthrene. 5-Methylcholanthrene, on the other hand, was significantly less potent than cholanthrene. Hydrogenation of the cholanthrene or 3-methylcholanthrene molecule resulted in a significant decrease in carcinogenicity of these compounds in regard to lung carcinoma induction.

4. It is concluded that a) cholanthrene and its derivatives act differently as carcinogens to different tissues, b) substitution of a methyl group may increase or may decrease carcinogenicity dependent upon the substitution position within the molecule. From the data obtained, under the conditions of this experiment, the position of the methyl group was found to be a more decisive factor in influencing the carcinogenicity of the cholanthrene molecule than its mere addition.

REFERENCES

1. Bachmann, W. E. The Reaction of Alkali Metals with Polycyclic Hydrocarbons: 1,2-Benzanthracene, 1,2,5,6-Dibenzanthracene and Methylcholanthrene. J. Org. Chem., **1**:347-353. 1936.
2. Bachmann, W. E., and J. M. Chemerda. The Synthesis of 4-Methylcholanthrene and 5-Methylcholanthrene. J. Org. Chem., **6**:50-53. 1941.
3. Cook, J. W., and G. A. D. Haslewood. The Synthesis of 5:6-Dimethyl-1:2-benzanthraquinone, a Degradation Product of Deoxycholic Acid. J. Chem. Soc., 428-433. 1934.
4. Cook, J. W., G. A. D. Haslewood, C. L. Hewett, I. Hieger, E. L. Kennaway and W. V. Mayneord. Chemical Compounds as Carcinogenic Agents. Am. J. Cancer, **29**:219-259. 1937.
5. Fieser, L. F., and M. S. Newman. Methylcholanthrene from Cholic Acid. J. Am. Chem. Soc., **57**:961. 1935.
6. Fieser, L. F., M. Fieser, E. B. Hershberg, M. S. Newman, A. M. Seligman, and M. J. Shear. Carcinogenic Activity of the Cholanthrenes and of Other 1:2-Benzanthracene Derivatives. Am. J. Cancer, **29**:260-268. 1937.
7. Fieser, L. F. Carcinogenic Activity, Structure, and Chemical Reactivity of Polynuclear Aromatic Hydrocarbons. Am. J. Cancer, **34**:37-124. 1938.
8. Shear, M. J. Studies in Carcinogenesis. III. Isomers of Cholanthrene and Methylcholanthrene. Am. J. Cancer, **28**:334-344. 1936.

Diet and Hepatic Tumor Formation*†

J. A. Miller, M.S., D. L. Miner, M.S., H. P. Rusch, M.D., and C. A. Baumann, Ph.D.

(From the Department of Biochemistry, College of Agriculture, and the McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison, Wis.)

(Received for publication July 5, 1941)

Introduction

Hepatic tumors readily appear in rats fed a basal ration of carrots and polished rice to which butter-yellow (N,N-dimethyl-p-aminoazobenzene) has been added in oil (12, 16), and tumor incidence can be markedly reduced when liver or yeast is added to such a ration at a 10 to 15 per cent level (16, 17) or when the basal ration is wheat (13, 20). Nutritionally the rice-carrot diet is grossly deficient in several dietary essentials such as protein, salts, and various members of the vitamin B complex. However, neither the addition of fish protein, nor salts, nor the known members of the vitamin B complex alone or in combination, prevented the formation of liver tumors (16). Recently the combination of high riboflavin and high casein has been reported to inhibit hepatoma formation on a diet of rice and carrot (11).

Our studies concern the effects of numerous modifications in the basal diet on the rate of tumor formation with butter-yellow. Semisynthetic diets adequate for growth and maintenance were employed and particular emphasis placed on the relative effectiveness of various proteins. Certain substances known to be present in liver and yeast were also assayed for protective potency. In the course of these investigations a laparatomy technic for the detection of early liver tumors was perfected.

Method

Liver tumors were induced in stock albino rats (125 to 200 gm.) by feeding butter-yellow [1] at a level of 0.06 per cent or more for a period of 120 days. The dye was incorporated in various rations by dissolving it with heat in the fat of the diets. Food and water were given ad libitum. The experiments were performed in a number of series in each of which the animals were divided into groups of 10 to 12 comparable in age and weight. All animals in a group were numbered and kept in a large single cage and the average intake of food and dye for the group determined at monthly intervals. The animals were weighed at least every two weeks during feeding of butter-yellow. At the end of this time laparotomies were performed and the livers inspected with the aid of a flat glass probe attached to a pencil flashlight. The animals were then sewed up and fed the basal diet without butter-yellow for an additional two months or more. During this time additional liver changes were detected by palpation and by a second laparotomy usually performed at 6 months. Thus tumors present but not recognized as such at 120 days were allowed to manifest themselves in the following 60 days.

In over 500 operations only 14 cases of postoperative adhesions and pneumonia were observed. Deaths due to overdoses of ether, or to surgical shock in animals in a poor nutritional state accounted for the 5 per cent mortality sustained. The liver changes were recorded as −, macroscopically normal; ±, uneven cirrhotic surface but otherwise normal; +, few nodules of "cholangioma" or "hepatoma" with or without an uneven cirrhotic surface; ++, extensive liver cancer with or without cirrhosis; +++, very large liver cancer with or without metastasis. By the term "cirrhosis" is meant a derangement of the liver by strands of proliferating fibrous tissue such that the liver presents a gross "hob-nailed" appearance.

With the exception of the stock ration all basal diets employed were of a purified nature and consisted of variations of the following:

	Per cent
Protein (casein, egg white, liver, yeast, etc.)	10, 12, 18, or 40
Cerelose [2] or dextrin, varied to suit protein level	
Cottonseed oil [3] or butter fat	5, 20, or 30
Salts	4
Cod liver oil	1 or 2

* This investigation was aided by grants from the Jonathan Bowman Fund for Cancer Research, the Wisconsin Alumni Research Foundation, and the Wisconsin Natural Science Project, W.P.A. No. 10305. The authors are also indebted to B. E. Kline for assistance in certain phases of the problem.

† Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[1] N,N-dimethyl-p-aminoazobenzene, No. 338, Eastman Kodak Co.

[2] A pure commercial glucose monohydrate.

[3] Fresh cottonseed oil sold under the trade name "Wesson Oil" or hydrogenated cottonseed oil sold under the trade name "Primex."

	Per cent
Vitamin B complex and protein as:	
Dried brewer's yeast [4]	1, 8, 31, or 51
Whole dried beef liver [5]	10 or 20
Water extracted beef liver [5]	7 or 19
Dried powdered grass [6]	10
Vitamin B complex as 1 or 4 drops of Vitab [7] per rat per day.	

In all, 34 different diets were fed.

Experimental

Tumor formation on an adequate stock diet.—In our first experiment an attempt was made to induce hepatomas with butter-yellow in rats receiving a nutritionally adequate diet (10) employed for many years as a stock ration in this laboratory. This diet had the following composition:

	Per cent
Corn meal	40
Soybean meal	10
Linseed oil meal	15
Alfalfa leaf meal	3
Powdered skim milk	20
NaCl (fine, iodized, 0.023 per cent KI)	1
$Ca_3(PO_4)_2$	1
Butter fat	10
	100

This mixture contained crude protein, 20 per cent; riboflavin 50 micrograms per 10 gm. (calc.).

The experiment was also designed to test the effect of subcutaneous injections of CCl_4 on the rate of liver tumor formation. The dose and frequency of the injections were such as to induce a mild reversible hepatic cirrhosis (5). Four groups of 10 albino rats (125 to 200 gm.) were fed the stock diet and treated as follows:

Group 1 = CCl_4 injections (0.1 cc. every 3 days for 18 days followed by a 10-day rest period) for 112 days
Group 2 = 0.06 per cent butter-yellow
Group 3 = 1 and 2 simultaneously
Group 4 = 1 for 56 days followed by 2.

Of the 30 rats receiving the dye, 17 were killed and examined after 4 months. None of them had visible tumors. This was in sharp contrast to the very high tumor incidence observed after 4 months when inadequate basal diets were fed (see below). Of the remaining 13 rats 10 developed liver tumors between 4 and 12 months of continuous butter-yellow ingestion, the greatest incidence occurring at 6 to 8 months (Table III, stock diet). Three of these animals were still alive and free from palpable tumors after 11 to 13 months of continued administration of the dye. In other words the stock diet had substantially delayed the production of tumors due to butter-yellow but did not prevent their ultimate appearance. No obvious effects of the CCl_4 were noted, tumor formation being retarded in all groups by the stock diet. However, the injections of CCl_4 prior to the administration of the butter-yellow may have increased the rate of tumor formation slightly. A tumorous female of group 4 bore and successfully raised a litter of 6. These young were placed at weaning on the stock diet plus 0.06 per cent butter-yellow. Their livers were free from tumors after 4 months of butter-yellow ingestion, again demonstrating the protective potency of the diet (Table III, stock diet). In fact carcinogenesis on the stock ration was very similar to that observed on inadequate diets fortified with 10 per cent of whole liver (see below), although the degree of protection was somewhat less on the grain ration. Thus the livers of animals fed butter-yellow plus whole liver were enlarged but otherwise entirely normal at 4 months, whereas on the stock diet they appeared less deeply colored and often possessed a fine surface granulation. At 6 months tumors on the stock diet were accompanied by increased granulation and typical precancerous white spots throughout the liver. On the contrary, the livers of rats fed liver appeared entirely normal even after 7 months of dye administration, although 2 tumors eventually formed in this group (Table III, diet 26).

Tumor production on purified diets.—In spite of all the information available on the nutritional requirements of the albino rat, the basal ration used in every reported dietary study of liver tumors has consisted of rice and carrot (11, 13, 16, 17, 20). The reasons advanced for the use of this diet are that liver tumors develop readily on such a regimen, and that the multiple deficiencies involved simulate the deficiences of the Oriental diet on which human liver cancer is not infrequent (6, 15). However, in our experience young rats fed a diet of carrots and polished rice grow very poorly even in the absence of butter-yellow, and then only when relatively large amounts of carrot are given. In the presence of the dye, food consumption is lowered still more, the animals lose weight, complications such as edema arise, and mortality is high.

The advantages of a purified diet for studies of this type are obvious. In devising one which would allow a high tumor incidence, two basal types were tested. The first represented a conscious attempt to reproduce

[4] Anheuser Busch, Strains C and G.

[5] The Wilson Laboratories, Chicago, Illinois.

[6] For this sample we are indebted to Dr. G. Kohler, Cerophyll Laboratories, Kansas City, Mo.

[7] "Vitab Rice Bran Concentrate," National Oil Products Co., Harrison, N. J. This substance is stated by the manufacturer to contain at least 150 micrograms/cc. (1.29 gm.) of thiamin; 10 micrograms/cc. (1.29 gm.) riboflavin; 100-150 micrograms/cc. (1.29 gm.) pyridoxine; 300-400 micrograms/cc. (1.29 gm.) pantothenic acid; and 1-2 mg./cc. (1.29 gm.) of nicotinic acid. By microbiological assay (Mr. R. Potter, Biochemistry Dept.) this substance contained 11 micrograms of riboflavin per gram (11).

the rice-carrot diet in terms of low protein and low vitamin levels. The other was designed to be low in protein but adequate for growth in all other respects. These diets contained the following constituents:

"Rice-carrot Model"	Per cent	Low Protein	Per cent
Crude casein	10	Crude casein	9-12
Dextrin	79	Cerelose	80-77
Salts	4	Salts	4
Butter fat	5	Cottonseed oil	5
Cod liver oil	1	Cod liver oil	2
Yeast	1	Vitab [7]	0.2 gm. (4 drops per rat per day)

The source of the vitamin B complex in the low protein diet, Vitab,[7] was a commercial aqueous rice bran extract. When young rats were fed 2 drops (0.1 gm.) per day as a source of the vitamin B complex in an otherwise adequate diet containing 18 per cent of crude casein, they gained 2.8 gm. daily in the absence of butter-yellow; when 4 drops per day were fed, growth averaged 3.5 gm. daily. Thus our Vitab-containing diet was adequate for growth in spite of the fact that the riboflavin content was lower than the usually accepted level of 10 micrograms per rat per day (Tables I to III).

As expected, growth on the deficient "rice-carrot model" diet was poor. The rats either just maintained their weights or declined slowly, and when butter-yellow was added, the decline was more rapid. Rats on the low protein diet (9 per cent casein) gained from 3 to 4 gm. per week in the absence of butter-yellow, and the addition of dye reduced growth to nearly zero. On 12 per cent casein weight and general health were maintained to a greater degree and this level was finally adopted for routine low-protein diets containing the dye. The mortality encountered with both diets plus butter-yellow amounted to 30 to 50 per cent on average casein levels of less than 12 per cent. Although high, the losses sustained were not so great as those reported by the Japanese (12, 16) who observed a general mortality of 60 to 90 per cent even in diets apparently containing higher levels of protein. When 12 per cent protein was fed to our animals throughout the experiment, the losses averaged 27 per cent. Sugiura and Rhoads (26) report a mortality of 5 per cent on high protein and only 15 per cent mortality on their diet of carrot and unpolished rice. On our diets deaths usually occurred within the first 2 months of butter-yellow feeding. Since the animals were kept in groups, cannibalism sometimes prevented inspection of the livers of those which died.

Tumor incidence at 4 months was 90 to 100 per cent on both of the semisynthetic diets (Table I, diets 1-4) and the livers were severely cirrhotic. The tumorous livers were in every respect similar to those described or photographed by Nakahara *et al.* (16, 17), Sugiura and Rhoads (26), Orr (22), and Kinosita (12), all of whom used the rice-carrot diet. Thus our results indicate that efficient tumor production with butter-yellow is possible on purified diets of controllable inadequacy.

Dietary factors which affect the development of liver tumors.—For purposes of comparison the many diets fed have been grouped into three master tables on the basis of their effectiveness in promoting or hindering the development of liver tumors. Diets which favored tumor formation are listed in Table I, those which partially retarded tumor formation in Table II, and those which markedly retarded tumor formation in Table III.

A study of these tables leads to certain general conclusions; *viz.,* 1. that the nutritionally adequate diets offered at least partial protection against hepatoma formation; 2. that the protective supplements were usually rich in both protein and the vitamin B complex; and 3. that the nonprotective diets were deficient in at least one of these factors.

Anticarcinogenic effects of yeast, of liver, and of liver fractions.—As examples of common food materials known to counteract butter-yellow when added to a rice-carrot diet (16, 17), yeast and liver were added to our semisynthetic diets and tested for their effectiveness against butter-yellow tumors. Brewer's yeast was fed at a level of 8 per cent in a diet containing 18 per cent of casein, and it was also fed as 31 per cent or 51 per cent of diets containing no other source of protein. These levels corresponded to 12 and 18 per cent of crude protein respectively in the diets as fed. Whole dried liver was fed at a level of 10 per cent in a diet containing 10 per cent of casein, and also as 20 per cent of a diet containing no other source of protein or of the vitamin B complex.

Tumor incidence was 40 per cent when 8 per cent of yeast was fed in addition to 18 per cent of casein (Table II, diet 17). This agrees with an incidence of 30 per cent reported by Sugiura and Rhoads (26) when 6 per cent of yeast was added to the rice-carrot diet. In other words this level of yeast afforded partial protection against butter-yellow. Much larger levels of yeast, however, failed to afford greater protection. When yeast at 31 and 51 per cent of the diet furnished both the sole source of protein and of the vitamin B complex, the incidence of hepatic tumors was 25 and 40 per cent respectively. Since only 15 per cent of yeast has been reported to furnish complete protection against butter-yellow when added to a rice-carrot diet (26) it would appear that the combination of rice and yeast protein is more efficient than much larger amounts of yeast protein alone. Certainly the supply of the vitamins B must have been more than adequate in the diet containing 51 per cent yeast.

TABLE I: DIETS WHICH FAVOR THE FORMATION OF LIVER TUMORS DUE TO BUTTER-YELLOW

Diet no.	Per cent casein (crude)	Supplement or other protein source and per cent	Per cent crude protein	Vitamin B complex	Riboflavin intake μgm./rat/day	Av. wt. increment in 4 mo. gm./rat	Av. daily food intake gm./rat	Av. butter-yellow intake in 4 mo. gm./rat	Survival [2] at 4 mo.	Deaths of negative animals between 4 and 6 mo.	Tumor incidence [3] 4 mo.	Tumor incidence [3] 6 mo.	Degree of cirrhosis	Comment
1	10	..	10	1% yeast	5	−23	7.5	0.54	10/10	Killed at 5 mo.	9/10 at 5 mo.	..	Severe	
2	10	..	10	4 Vitab [1]	5	+ 6	7.0	0.67	7/10	0	7/7	..	Severe	4 killed at 4 mo.
3	12	..	12	4 Vitab	5	−21	7.5	0.72	7/11	0	7/7	..	Severe	
4	10	..	10	2 Vitab	3	−10	6.0	0.39	14/30	5	7/14	10/14	Severe	Off dye for 12 days
5	10	10—dried grass	10+	1% yeast	25	−12	10.0	0.72	10/10	Killed at 5 mo.	9/10 at 5 mo.	..	Severe	
6	10	5—grass juice	10+	1% yeast	5	−33	9.0	0.65	10/10	Killed at 5 mo.	9/10 at 5 mo.	..	Severe	
7	12	0.5—l-cystine	12	4 Vitab	5	−15	8.0	0.77	9/11	0	8/9	9/9	Severe	
8	12	0.5—i-inositol	12	4 Vitab	5	−19	6.0	0.58	6/12	0	6/6	6/6	Severe	
9	18	0.5—i-inositol	18	4 Vitab	5	+16	7.0	0.67	12/12	0	11/12	11/12	Slight	1 "+" liver at 4 mo. was "−" at 6 mo.
10	10	1—urea	10	2 Vitab	3	−16	4.1	0.22	8/10	0	4/8	..	Severe	Off dye for 3 wks.

[1] "Vitab Rice Bran Concentrate": 1, 2, or 4 drops per rat per day.
[2] "ALR," an alcohol liver residue, *i.e.*, that portion of the water-soluble material of whole liver that is insoluble in 70 per cent alcohol.
[3] Survival is recorded as the number living at 4 months over the number of animals at the beginning. Tumor incidence is recorded as the number with tumors over the number surviving at 4 months.
[4] BY = butter-yellow.

TABLE II: DIETS WHICH PARTIALLY RETARD THE FORMATION OF LIVER TUMORS DUE TO BUTTER-YELLOW *

Diet no.	Per cent casein (crude)	Supplement or other protein source and per cent	Per cent crude protein	Vitamin B complex	Riboflavin intake μgm./rat/day	Av. wt. increment in 4 mo. gm./rat	Av. daily food intake gm./rat	Av. butter-yellow intake in 4 mo. gm./rat	Survival [2] at 4 mo.	Deaths of negative animals between 4 and 6 mo.	Tumor incidence [3] 4 mo.	Tumor incidence [3] 6 mo.	Degree of cirrhosis	Comment
11	18	..	18	1 Vitab	2	25	8	0.58	9/10	2	2/9	4/9	Slight	
12	18	..	18	4 Vitab	5	110	10	0.72	8/10	2	2/8	4/8	Slight	
13	18	0.3—choline	18	1 Vitab	2	35	8	0.58	9/10	1	3/9	6/9	Slight	
14	20	30—fat	20	2 Vitab	4	20	6	0.54	9/10	3	1/9	5/9	Slight	Low food intake, fed dye for 5 mo.
15	10	20—fat	10	2 Vitab	3	−27	5.6	0.34	9/11	3	2/9	3/9	Severe	Low food intake, off dye for 3 wks.
16	40	..	40	1 Vitab until 2nd mo., then 2	5	73	6—1st 2 mo. 10—2nd 2 mo.	0.58	7/10	0	0/7	5/7	None	6/7 by 8 mo.
17	18	8 yeast	21	8% yeast	40	160	11.5	0.83	10/10	0	4/10	5/10	None	
18	..	31—yeast	12	31% yeast	108	44	7	0.67	8/12	0	2/8	4/8	None	"Infiltrative" tumors
19	..	51—yeast	18	51% yeast	203	33	8	0.77	10/12	0	4/10	4/10	None	"Infiltrative" tumors
20	..	12—egg white	12	4 Vitab	7	50	8	0.77	9/12	0	5/9	5/9	None	"Infiltrative" tumors
21	..	19—water extracted liver	12	4 Vitab	20	−14	8.5	0.82	11/11	1	2/11	4/11	Slight	
22	10	7—water extracted liver	14	1% yeast	12	108	12.8	1.15	10/10	Killed at 5 mo.	5/10 at 5 mo.	..	None	
23	10	8—ether extracted liver	15	1% yeast	12	147	13.0	1.17	8/10	Killed at 5 mo.	2/8 at 5 mo.	..	None	
24	10	Ether extract of liver 20—liver	10	1% yeast	4	− 2	8.5	0.77	9/10	Killed at 5 mo.	3/9 at 5 mo.	..	Medium	
25	10	0.1—xanthine	10	1% yeast	4	−22	7.0	0.63	11/11	Killed at 5 mo.	5/11 at 5 mo.	..	See comment	6—very small yellow and cirrhotic

* For explanatory footnotes see Table I.

TABLE III: DIETS WHICH MARKEDLY RETARD THE FORMATION OF LIVER TUMORS DUE TO BUTTER-YELLOW *

Diet no.	Per cent casein (crude)	Supplement or other protein source and per cent	Per cent crude protein	Vitamin B complex	Riboflavin intake μgm./rat/day	Av. wt. increment in 4 mo. gm./rat	Av. daily food intake gm./rat	Av. butter-yellow intake in 4 mo. gm./rat	Survival # at 4 mo.	Deaths of negative animals between 4 and 6 mo.	Tumor incidence # 4 mo.	Tumor incidence # 6 mo.	Degree of cirrhosis	Comment
Stock	7	Yellow corn—40; skim-milk pwd. —20; linseed oil meal—15	20	See supplements	50	120	12	0.88	30/30	17 killed at 4 mo.	1 3 at 7-8 mo. 2 at 12 mo.	4	Slight	3 alive without tumors after 11-13 mo. continuous dye feeding.
Stock	7	Alfalfa leaf meal —3	20	See supplements	34	140	8	0.59	6/6	..	0/6	..	None	Received dye from weaning until 4 mo., 3 wks. of age.
26	10	10—liver	16	10% liver	100	100	10	0.72	9/10	2	0/9	0/9	None	Continuous dye feeding. 2 palpable tumors at 7 mo.
27	10	10—liver	16	1 Vitab 10% liver	100+	73	10.5	0.76	10/10	2	0/10	0/10	None	
28	10	10—liver	16	2 Vitab 10% liver	100+	151	12.5	1.13	10/10	Killed at 5 mo.	1/10 at 5 mo.	..	None	
29	..	20—liver	13	1% yeast 20% liver	140	33	7	0.67	9/12	0	1/9	1/9	None	
30	10	2—water sol., alcohol insol. liver residue (ALR) [2]	11	2 Vitab	25	0	6.8	0.55	7/10	3	1/7	4/7	None	
31	10	1.2—ALR [2]	10.5	1% yeast	22	51	9.8	0.88	11/11	Killed at 5 mo.	2/11 at 5 mo.	..	None	
32	10	1.5—"water sol." ALR	10.4	2 Vitab	15	−36	5.0	0.30	8/10	7	0/8	0/8	None	Off dye 3 wks
33	10	0.5—"water insol." ALR	10	2 Vitab	8	27	6.8	0.49	9/10	0	1/9	6/9	Slight	
34	10	20—fat 2—ALR	10	2 Vitab	24	0	6.6	0.48	9/11	0	0/9	1/9	None	1 ± at 4 months.

* For explanatory footnotes see Table I.

TABLE IV: THE EFFECT OF BUTTER-YELLOW ON THE GROWTH OF RATS WITH AND WITHOUT CONTROLLED FOOD INTAKE *

Diet	Per cent casein (crude)	Source of Vitamin B complex	Other Supplement	Feeding	Butter yellow 0.06 per cent	Av. initial wt. gms.	Av. wt. increment gm./rat/day	Av. food consumption gm./rat/day	Maximum per se effect of BY [4] on growth per cent	Per cent change in food consumption	Comment
1	18	8% yeast	..	Ad lib.	−	62	4.2	9.3	..	..	
1	18	8% yeast	..	Ad lib.	+	53	1.4	6.7	..	−27	
1	18	8% yeast	..	Controlled	−	62	2.2	6.4	−35	..	
2	18	2 Vitab [3]	..	Ad lib.	−	66	2.8	8.8	..	..	
2	18	2 Vitab	..	Ad lib.	+	54	0.6	5.3	..	−40	
2	18	2 Vitab	..	Controlled	−	63	0.5	5.3	+16	..	
3	10	2 Vitab	..	Ad lib.	−	37	0.85	6.8	..	..	
3	10	2 Vitab	0.5% l-cystine	Ad lib.	−	40	3.0	6.0	..	..	
3	10	2 Vitab	0.5% l-cystine	Ad lib.	+	36	0.45	4.0	..	−33	Edema in 3 weeks.
4	12	4 Vitab	..	Controlled	−	36	0.80	3.8	..	..	
4	12	4 Vitab	1.0% l-cystine	Controlled	−	36	0.85	3.8	..	..	
4	12	4 Vitab	..	Ad lib.	+	40	0.5	3.8	−38	..	Slight edema in 3 weeks.
4	12	4 Vitab	1.0% l-cystine	Controlled	+	39	0.5	3.8	−43	..	
5	18	4 Vitab	..	Controlled	−	34	0.75	3.0	..	..	
5	18	4 Vitab	..	Ad lib.	+	37	0.55	3.0	−27	..	Edema by 16th day.
5	18	4 Vitab	0.5% l-cystine	Controlled	+	36	0.35	3.0	..	..	No edema.
6	18	4 Vitab	..	Controlled	−	43	0.80	3.8	..	..	
6	18	4 Vitab	0.5% l-cystine	Controlled	−	44	1.25	3.8	..	..	
6	18	4 Vitab	..	Ad lib.	+	47	1.10	3.8	−38 †	..	Edema by 14th day.
6	18	4 Vitab	0.5% l-cystine	Controlled	+	46	0.80	3.8	−36	..	No edema.

* For explanatory footnotes see Table I.

† See Comment.

In agreement with results of others (16) we found that 10 per cent whole liver completely protected our animals against the development of tumors after 4 months of butter-yellow feeding (Table III, diets 26 and 27). Nearly complete protection was observed after 5 months of administration of the dye (Table III, diet 28), but after 7 months of continuous administration tumor incidence was 22 per cent in one group (Table III, diet 26). One tumor developed during 6 months of feeding 20 per cent of liver as the sole source of protein (12 per cent) (Table III, diet 29). This demonstrated that the level of protein needed for protection is not particularly high provided other protective factors are fed in adequate amounts.

Certain compounds known to be in liver were then tested in pure form for possible anticarcinogenic activity. Xanthine was tested because it has been reported to protect against hepatic cirrhosis as induced by CCl_4 or $CHCl_3$ (9). Butter-yellow also induces severe cirrhosis on a low-casein diet. Cystine seems to enable the animal organism more easily to detoxify carcinogenic compounds, including the azo dyes (27, 28). One of the functions of choline is the prevention of fat deposits in the liver due to high dietary fat or to pancreatectomy (3, 4). Inositol has recently been proposed as a dietary essential for the mouse (30). These compounds occur in dried whole liver to the following extent: xanthine, approximately 0.08 per cent isolated from pork liver (19); l-cystine, 0.68 per cent in beef liver (21); choline, 0.27 per cent in beef liver (8); i-inositol approximately 0.01 per cent isolated from beef liver (31).

It was accordingly possible that any one of these compounds might have been a limiting factor under the conditions imposed by butter-yellow feeding.[5] However, when these substances were added singly to butter-yellow diets at levels above those supplied by 10 per cent of whole liver, none was found to exert any marked protective effect (Tables I and II, diets 7 to 10) against hepatoma formation. On the contrary, 0.5 per cent i-inositol added to an 18 per cent casein diet actually increased tumor incidence as compared to the basal diet alone (Table I, diet 9).

Certain liver extracts and extraction residues were also fed. An ether extract of liver made at 35° C. allowed a tumor incidence of 33 per cent at levels equivalent to 20 per cent of whole liver (Table II, diet 24). Sugiura (26) has also reported protection with fat-soluble material, an ether extract of rice bran. When the ether-insoluble residue of liver was fed at a level of 8 per cent the incidence of tumors was 25 per cent at 5 months (Table II, diet 23). The residue from an exhaustive aqueous extraction of whole liver at 70° C. was fed as (a) 7 per cent ($\cong$ 10 per cent of whole liver) of a diet also containing 10 per cent of casein, and (b) 19 per cent of a diet ($\cong$ 12 per cent total protein) containing no other protein source. The incidence of tumors on the diet containing 7 per cent liver residue was 50 per cent at 5 months (Table II, diet 22), whereas on 19 per cent liver residue, 18 and 36 per cent of the animals had tumors at 4 and 6 months respectively (Table II, diet 21). In other words these fractions exerted partial protection against butter-yellow in amounts which furnished only moderate amounts of total protein.

The most potent liver fraction was found to be the water-soluble, alcohol-insoluble portion. This material was supplied by Dr. David Klein of the Wilson Laboratories and consisted of the precipitate obtained when the aqueous extract of whole liver was treated with ethyl alcohol until a concentration of 70 per cent alcohol was reached. On levels of 1.2 and 2 per cent of this fraction ($\cong$ 10 and 17 per cent whole liver) in diets containing 10 per cent of casein, tumor incidence was only 0 to 18 per cent; *i.e.*, the degree of protection ranged from 82 to 100 per cent (Table III, diets 30, 31, 34). The material as supplied was further divided into a water-soluble (60 per cent) and a water-insoluble fraction (40 per cent), and each was fed at low levels. Inconclusive results were obtained with the water-soluble fraction at a level of 1.5 per cent, for the dye was removed from the diet for 3 weeks due to the poor condition of the animals, and 7 of these died between the 4th and 6th month (Table III, diet 32). The water-insoluble fraction at a level of only 0.5 per cent not only reduced the incidence of tumors to 13 per cent at 4 months (Table III, diet 33) but even permitted a gain in weight on the low protein diet plus butter-yellow. The protective potency of the fraction, however, was considerably below that of 10 per cent of whole liver, since the tumor incidence at 6 months ranged from 11 to 66 per cent (Table III, diets 30 to 34). Nevertheless the degree of inhibition caused by these small amounts of liver fractions again demonstrated that a level of dietary protein above the accepted nutritional standard was not necessary for protection against butter-yellow provided the proper anticarcinogens were furnished either in the protein or otherwise.

These alcohol-insoluble liver fractions represent the most potent concentrates of natural protective factors

[5] György, P., E. C. Poling, and H. Goldblatt, in a recent paper entitled: Necrosis, Cirrhosis and Cancer of Liver in Rats Fed a Diet Containing Dimethylaminoazobenzene, published in Proc. Soc. Exper. Biol. & Med., **47**:41-44. 1941, report that "the combined oral administration of cystine plus choline afford a definite but not regular protection against pathological changes in the liver (necrosis, cirrhosis, atypical nodular proliferation of bile ducts, adenocarcinoma, malignant hepatoma) produced by a diet containing dimethylaminoazobenzene."

yet reported. Further fractionation is in progress. The riboflavin content of these fractions was determined fluorometrically. The average values obtained were: original alcohol-insoluble fraction, 180 μgm. per gm; water soluble fraction, 140 μgm. per gm., and water insoluble fraction, 110 μgm. per gm. The very high riboflavin content of these liver fractions assumes considerable importance in light of the recent report by Kensler and associates (11).

The effect of protein.—While the preceding experiments appeared to indicate that protein in itself was not anticarcinogenic to butter-yellow, it was recognized that the protein fractions fed (yeast, whole liver, liver extract, liver residue) were all rich in nonproteinaceous substances which might have exerted considerable influence on the end result. Accordingly various levels of casein and egg white were fed for comparison. Casein was fed at 10, 12, 18, and 40 per cent of the diet; egg white at 12 per cent.

The incidence of hepatic tumors was found to decrease as the level of protein was increased. Thus on 10 or 12 per cent of casein, tumor incidence was 90 to 100 per cent at 4 months (Table I, diets 1 to 3). On 18 per cent casein tumor incidence was only 23 per cent at 4 months and 47 per cent at 6 (Table II, diets 11 and 12). On 40 per cent casein no tumors had appeared by 4 months, but the incidence had increased to 71 per cent by 6 (Table II, diet 16). Egg white seemed to exert more protection against butter-yellow than casein. When 12 per cent of egg white constituted the sole source of protein in the diet, tumor incidence was 55 per cent at both 4 and 6 months (Table II, diet 20) as contrasted with 90 to 100 per cent incidence when casein was fed at this level. Referring again to the previously discussed results with yeast and liver, tumor incidence at an equivalent of 12 per cent crude protein was 25 per cent at 4 months and 50 per cent at 6 for yeast; and 18 per cent at 4 months and 36 per cent at 6 for the water-extracted liver.

The effects of some of these proteins were more marked than the figures indicate. Thus on casein levels of 18 per cent "hobnail cirrhosis" was almost entirely absent, and on the other proteins it did not appear at levels as low as 12 per cent. Furthermore the gross character of the tumors appeared to depend on the nature of the protein fed. Thus on egg white or on high yeast the tumors were of an "infiltrative" type, in which the tumor tissue had replaced liver tissue within the normal confines of the organ and failed to form any surface nodules. Usually these had grown outward from the liver lobe by 6 months, and in some animals entire lobes had become white by this time. Microscopic examination confirmed the tumorous nature of these livers.

Our results with protein can therefore be summarized with the statement that protection against butter-yellow is possible in the presence of as little as 12 per cent of certain proteins, that an increase in the level of a relatively nonprotective protein like casein reduces tumor incidence, and that certain protein sources such as egg white and extracted liver exert a greater degree of protection than casein. Nakahara reported that 10 per cent of fish protein added to the rice-carrot diet failed to reduce tumor incidence (16). Sugiura and Rhoads (26) observed no protection when casein was added to the rice-carrot diet although the nutritional state of their animals was markedly improved by the addition. Kensler and associates (11) observed no improvement with either casein alone or riboflavin alone, but a marked improvement with the two together.

The question might be raised whether the anticarcinogenic action of protein is exerted through the suppression of cirrhosis. Since spontaneous liver tumors are often associated with cirrhosis, the generalization has been made that cirrhosis is a necessary prerequisite for hepatoma formation (7, 29), and this would appear to be supported by the almost universal development of cirrhosis on the rice-carrot diet and on our deficient semisynthetic diets containing 12 per cent of casein or less. However, when other sources of protein were fed, either alone or as supplements, hobnail cirrhosis was extremely rare, and the appearance of tumors without gross cirrhosis became the rule rather than the exception (Tables I-III). If cirrhosis is a necessary preliminary step in carcinogenesis, it must have been either extremely mild or very much localized in the latter instances.

In earlier studies the presence of cirrhotic lumps often made it virtually impossible to determine whether a given liver was tumorous or not. This difficulty has been successfully overcome by withdrawing butter-yellow from the diet after 4 months. Even on diets containing 12 per cent of casein or less, hobnail cirrhosis regressed in the absence of butter-yellow to such an extent that the liver surface became smooth. True tumors, of course, continued to grow in the interim, and hence a second examination definitely established the presence or absence of tumors. Orr (22) also has reported a "regression" of "cirrhosis" due to butter-yellow.

The effect of variations in the vitamin B complex.—A role of the vitamin B complex in the development of liver tumors might be suspected from the fact that all substances which retard these tumors contain large amounts of these vitamins. However, Nakahara has reported that "nutritionally adequate" amounts of thiamin, pyridoxine, nicotinic acid, riboflavin, or a vitamin L concentrate, either alone or in combination with fish protein failed to alter tumor incidence on a

basal diet of rice and carrot (16). Variations in the vitamin B complex within the nutritional range likewise failed to alter tumor incidence in our present experiments. Thus, in the presence of 12 per cent of casein the number of tumors which developed was essentially the same whether 2 or 4 drops of rice bran concentrate were fed daily or whether the vitamins were supplied in 1 per cent of yeast (Table I, diets 1 to 4). Similarly in the presence of 18 per cent of casein the level of rice bran extract failed to alter tumor incidence (Table II, diets 11 and 12). When very high amounts of the B vitamins were fed as 51 per cent of yeast (=18 per cent total protein), protection against butter-yellow was still incomplete (Table II, diet 19).

However, Kensler *et al.* (11) report that supplements of riboflavin and casein prevented butter-yellow tumors in rats receiving a basal diet of rice and carrot. Accordingly our diets were examined for their contents of riboflavin and protein (Tables I to III). The approximate riboflavin intakes were calculated from the riboflavin content of the crude casein, yeast, and Vitab supplements employed. As a result it appears that the diets which favored tumor formation (Table I) were low in both protein and riboflavin. Diets that partially retarded tumor formation (Table II) contained more casein, or protein of better quality, but they were generally low in riboflavin. The diets which markedly retarded tumor formation (Table III) contained much riboflavin in addition to sufficient protein or other protective supplements. Thus our results, like those of Kensler *et al.* (11) indicate that the effects of butter-yellow may be reduced by diets high in riboflavin and protein. These investigators fed 200 μgm. of riboflavin plus 2 grams of casein daily in addition to a basal diet of rice and carrot and observed a tumor incidence of only 6 per cent at 4 months, at which time the animals were sacrificed. It is quite possible, however, that a higher tumor incidence would have been found at 6 months. Furthermore, from our data (Table III, stock diet and diets 31, 33), it would appear that the minimum amount of riboflavin needed for nearly complete protection at 4 months is probably not much above 25 μgm. per day provided enough other necessary factors are present. This amount of riboflavin is at the most only about 5 times the minimum needed for good growth in the albino rat.

In our diets the Vitab supplement at 4 drops (0.2 gm.) per rat per day supplied 30 μgm. of thiamin, 24 μgm. of pyridoxine, 70 μgm. of pantothenic acid, and 300 μgm. of nicotinic acid. These levels are roughly 2 to 3 times the normal requirements of the first three compounds. Hence the question remains whether increased levels of other members of the vitamin B complex would not also exert an effect similar to that found for riboflavin. In other words protection against butter-yellow may not be specific for the combination of riboflavin and protein but may also result when high levels of other members of the vitamin B complex are fed in diets containing merely the ordinary requirements of all other dietary essentials. Our present results cannot be used to test this, since the limiting factor in many of our diets was riboflavin. Hence until such protection is demonstrated, the combination of riboflavin and the unknown factor or factors associated with protein must be regarded as the most specific anticarcinogen for butter-yellow reported to date.

Growth and Edema

Some indication of the mode of action of butter-yellow might be expected from a study of the physiological effects of the dye other than the carcinogenic response itself. Accordingly the growth and the food intake of animals 60 to 130 grams in weight was measured on each of the diets fed in the preceding studies, both in the presence and absence of butter-yellow. This also automatically revealed the nutritional adequacy of the various rations in the absence of the dye. The data thus collected indicated that the presence of 0.06 or 0.08 per cent of butter-yellow in the diet lowered the food consumption from 3 to 40 per cent (average, 21 per cent) and caused a reduction in growth rate of 21 to 100 per cent (average, 54 per cent). Since the effect on growth was greater than that on food intake, an effect of the dye on growth itself was suggested. This was verified in experiments involving controlled food intakes. The various diets fed and the combination of butter-yellow, cystine, and vitamin B complex fed are indicated in Table IV. Young growing rats 50 to 65 grams in weight, or weanlings of 30 to 40 grams were used. In any series litter mates were distributed equally among groups, and each group consisted of 2 rats. The feeding was continued for 2 to 3 weeks.

The results are summarized in Table IV. In nearly every series growth was less in animals ingesting butter-yellow than in comparable animals receiving the same amount of food but no butter-yellow. The decreases in growth due to the dye averaged 36 per cent. A complicating effect throughout the experiment, however, was the development of edema in most of the young animals receiving butter-yellow. This could not be attributed to the low level of food ingested, for control animals whose intake of food (and protein) were similarly restricted, developed no edema. A role of protein was nevertheless suggested by the fact that edema was reduced when cystine was fed in addition to butter-yellow (Table IV, series 4, 5, and 6). Cystine, however, did not increase the growth rate in the presence of butter-yellow provided the food intake

was restricted (Table IV, series 4, 5, and 6) although it markedly increased growth on low protein diets in the absence of the dye (Table IV, series 3).

Discussion

An anticarcinogen can be regarded as functioning in either of two ways: by counteracting the intracellular carcinogenic changes themselves, or by detoxifying or destroying the carcinogen before it is able to act. There are two reasons for suspecting that the latter mechanism is important in the case of butter-yellow tumors. The first, as Nakahara (16) has pointed out, is that the degree of dietary inhibition of these tumors is very much greater than that reported for other types. Thus, tumors due to tar-painting (14), to ultraviolet light (1), or to the local application of purified hydrocarbons (2) can be retarded only slightly when liver or a brain concentrate is fed. Tumors due to injected hydrocarbons have been retarded slightly by certain fat fractions (24). This difference might suggest that the relatively spectacular dietary inhibition of butter-yellow tumors has less to do with the actual carcinogenic process than the milder dietary response observed with the other carcinogens.

Secondly, the butter-yellow molecule offers more opportunity for destruction by the organism than the carcinogenic hydrocarbons. On reduction it can yield aniline and N,N-dimethyl-p-phenylene diamine; on demethylation it can yield p-aminoazobenzene. None of these compounds is carcinogenic (23, 12). Whatever the pathway involved, it is clear that the azo linkage is none too stable in the body, since the dye is not stored as such, and only minute amounts of yellow pigment are excreted (18). The chief function of the dietary anticarcinogen may therefore be merely to hasten the destruction of the carcinogen.

In the present study the usual method of producing liver tumors with azo dyes has been modified in two essential ways: purified synthetic diets were fed and a laparotomy technic was employed for the detection of tumors. The laparotomy not only avoided the wasteful sacrifice of experimental animals but aided in evaluating the carcinogenic state of animals with negative or questionable livers after 4 months. This was true since any cancerous change, even though hidden or doubtful, was allowed to develop into a frank unmistakable tumor during the subsequent period of 2 months when no more dye was fed. The second examination frequently revealed "late" tumors (Tables I to III). The new technic also offered a means of observing other effects of the dye, such as cirrhosis. Since "hobnail cirrhosis" usually regressed when the dye was withdrawn from the diet, this complicating factor in recognizing true tumors was also eliminated at the second examination. Apparently the operation itself neither hastened nor retarded the development of tumors.

Summary and Conclusions

1. Liver tumors were produced in rats by feeding butter-yellow in semisynthetic diets of controllable inadequacy for 4 months followed by the basal dye-free diet for 2 months more. The livers were inspected by laparotomy at 4 and 6 months. A tumor incidence of 90 to 100 per cent in 4 months was observed on diets containing 10 to 12 per cent casein but adequate in all other respects.

2. Dietary casein at levels of 18 to 40 per cent offered partial protection against butter-yellow. Other protein sources as liver, yeast, and egg offered equivalent protection at levels of 12 to 13 per cent of total protein. Whole dried brewer's yeast when fed at 31 to 51 per cent levels as the sole source of protein offered only partial protection.

3. Whole dried beef liver at a 10 per cent level in a 10 per cent casein diet or at a 20 per cent level as the sole source of protein offered nearly complete protection at 4 and 6 months. Two tumors were found at 7 months when the dye was fed continuously in a diet containing 10 per cent of liver.

4. The water-soluble, alcohol-insoluble portion of whole liver was found to offer nearly complete protection at 4 months when added at 1.2 to 2 per cent levels in carcinogenic diets containing 10 to 12 per cent of casein. However, protection was incomplete at 6 months. Xanthine, l-cystine, i-inositol, and choline at 0.1 to 0.5 per cent levels in carcinogenic diets did not offer any protection against the dye.

5. The food consumption of young rats receiving butter-yellow was reduced 21 per cent. Reductions in growth rate averaged 54 per cent. Controlled feeding experiments indicated that the dye itself reduced growth *per se* as well as through a decreased food intake.

6. Results with 34 diets indicated that in general, (a) the nutritionally adequate diets offered at least partial protection against hepatoma formation; (b) the protective supplements were usually rich in both protein and the vitamin B complex, particularly riboflavin; (c) the nonprotective diets were deficient in at least one of these factors.

References

1. Baumann, C. A., and H. P. Rusch. The Effect of Diet on the Production of Tumors with Ultra-Violet Light. Am. J. Cancer, **35**:213-221. 1939.
2. Baumann, C. A., H. P. Jacobi, and H. P. Rusch. The Effect of Diet on Experimental Tumor Production. Am. J. Hyg., **30**:1-6. 1939.

3. Best, C. H., and J. M. Hershey. Further Observations on the Effects of Some Component of Crude Lecithine on Depancreatized Animals. J. Physiol., **75**:49-55. 1932.
4. Best, C. H., J. M. Hershey, and M. E. Huntsman. The Effect of Lecithine on Fat Deposition in the Liver of the Normal Rat. J. Physiol., **75**:56-66. 1932.
5. Cameron, G. R., and W. A. E. Karunaratne. Carbon Tetrachloride Cirrhosis in Relation to Liver Regeneration. J. Path. & Bact., **42**:1-21. 1936.
6. De Leon, W. Malignancy Among the Filipinos, Trans. 7th Congress. Far Eastern Assoc. Trop Med., **1**:91-98. 1927. Cited by Sugiura, K., and C. P. Rhoads. Cancer Research, **1**:3-16. 1941.
7. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 4th Ed. p. 738. 1940.
8. Fletcher, J. H., C. H. Best, and O. M. Solandt. The Distribution of Choline. Biochem. J., **29**:2278-2284. 1935.
9. Forbes, J. C. Prevention of Liver Cirrhosis by the Subcutaneous Injection of Xanthine-Containing Preparations. J. Pharmacol. & Exper. Therap., **65**:287-293. 1939.
10. Jacobi, H. P., and C. A. Baumann. The Effect of Fat on Tumor Formation. Am. J. Cancer, **39**:338-342. 1940.
11. Kensler, C. J., K. Sugiura, N. F. Young, C. R. Halter, and C. P. Rhoads. Partial Protection of Rats by Riboflavin with Casein Against Liver Cancer Caused by Dimethylaminoazobenzene. Science, **93**:308-310. 1941.
12. Kinosita, R. Special Report. Studies on the Carcinogenic Chemical Substances. Tr. Soc. path. jap., **27**:665-727. 1937.
13. Kinosita, R. On the Substances to Affect the Experimental Carcinogenesis. Gann, **33**:225-229. 1939.
14. Maisin, J., and Y. Pourbaix. Growth Promoting and Inhibiting Substances Extracted from Normal Tissues. Am. J. Cancer, **24**:357-385. 1935.
15. Nagayo, M. Statistical Studies on Cancer in Japan. Gann, **27**: Extra Number, December, 1-176. 1933. Cited by Sugiura, K., and C. P. Rhoads, Cancer Research, **1**:3-16. 1941.
16. Nakahara, W., K. Mori, and T. Fujiwara. Inhibition of Experimental Production of Liver Cancer by Liver Feeding. A Study in Nutrition. Gann, **33**:406-428. 1939.
17. Nakahara, W., T. Fujiwara, and K. Mori. Inhibiting Effect of Yeast Feeding on the Experimental Production of Liver Cancer. Gann, **33**:57-65. 1939.
18. Nakamura, H., M. Yamada, H. Okamura, Y. Yoda, and T. Nishi. Experimentelle Studien über die intravenöse Anwendung des Buttergelbs und o-Amidoazotoluols. Gann, **32**:245-250. 1938.
19. Neale, R. C., and H. C. Winter. The Identification of the Active Crystalline Substance from Liver which Protects against Liver Damage Due to Chloroform or Carbon Tetrachloride; and a Study of Related Compounds. J. Pharmacol. & Exper. Therap., **62**:127-148. 1938.
20. Okada, D. Über den Einfluss der Brot-fütterung auf die Leberkarzinomentstehung durch Dimethylaminoazobenzol. Osaka Igaku Zasshi, **39**:485-488. 1940. Cited by Sugiura, K., and C. P. Rhoads. Cancer Research, **1**:3-16. 1941.
21. Okuda, Y. Quantity of Cysteine in Living Tissue Proteins and its Biological Significance. Proc. Imp. Acad. (Japan), **5**:246-248. 1929.
22. Orr, J. W. The Histology of the Rat's Liver During the Course of Carcinogenesis by Butter-yellow. J. Path. & Bact., **50**:393-408. 1940.
23. Sasaki, T., and T. Yoshida. Experimentelle Erzeugung des Lebercarcinoms durch Fütterung mit o-Amidoazotoluol. Virchows Archiv. f. path. Anat., **295**:175-200. 1935.
24. Shear, M. J., and J. Leiter. Tumor Production in Mice by Low Doses of Carcinogenic Hydrocarbons. Proc. American Society of Biological Chemists. XXXV:CXV. 1941.
25. Snell, E. E., and F. M. Strong. A Microbiological Assay for Riboflavin. Indust. & Engin. Chem., Anal. Ed., **11**:346-350. 1939.
26. Sugiura, K., and C. P. Rhoads. Experimental Liver Cancer in Rats and Its Inhibition by Rice-Bran Extract, Yeast and Yeast Extract. Cancer Research, **1**:3-16. 1941.
27. White, J., and A. White. Inhibition of Growth of Rats by Oral Administration of Methylcholanthrene. Effects of Dietary Cystine and Methionine Supplements. Proc. Soc. Exper. Biol. & Med., **39**:527-529. 1938.
28. White, J. Retardation of Growth of the Rat Ingesting p-Dimethylaminoazobenzene (Butter-Yellow) I. The Effect of Various Dietary Supplements. J. Nat. Cancer Inst., **1**:337-342. 1940.
29. Winternitz, M. C. Primäres Leberkarzinom. Virchows Arch. f. path. Anat., **209**:239-272. 1912.
30. Woolley, D. W. The Nature of the Anti-Alopecia Factor. Science, **92**:384-385. 1940.
31. Woolley, D. W. Identification of the Mouse Antialopecia Factor. J. Biol. Chem., **139**:29-34. 1941.

Failure of Choleic Acids of Carcinogenic Hydrocarbons to Alter Permeability of Marine Eggs and of Mammalian Erythrocytes*

Balduin Lucké, M.D., Arthur K. Parpart, Ph.D., and R. A. Ricca, M.D.

(From the Laboratory of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pa. the Laboratory of Physiology, Princeton University, Princeton, N. J., and The Marine Biological Laboratory, Woods Hole, Mass.)

(Received for publication June 30, 1941)

A considerable number of chemical substances of known composition are now available which on application to tissues induce cancer in certain species of vertebrates. The mechanism of the carcinogenic effect is not known. As cancer cells have been stated to differ from normal cells in their permeability (5, 7, 8), it seemed possible that carcinogenesis might involve alteration of surface properties, including cellular permeability.

The carcinogenic effect is not cell specific; that is, the same agent may act on a number of different types of cells and produce a variety of neoplasms. It therefore seemed likely that if one of the primary effects of a carcinogenic substance is to alter permeability, this much of its effect would be exerted on cells in general, although it is recognized that such an effect might be limited to cells capable of becoming malignant. It should be possible to detect such an alteration in those cells in which permeability may be measured accurately. At present the only animal cells available for such measurements are certain marine egg cells and mammalian erythrocytes.

Permeability is a universal property of living cells by means of which the diffusion of water and of dissolved substances into and out of cells is limited and regulated. Quantitatively, this property may be defined as the amount of a given substance which passes in either direction through a unit of cell surface, in a unit of time, as the result of a unit of force. In the present experiments the cells used were eggs of the sea urchin, *Arbacia punctulata,* and of the annelid, *Chaetopterus pergamentaceus,* and erythrocytes of mouse and beef. The carcinogenic agents were choleic acid compounds of 20-methylcholanthrene, 1,2,5,6-dibenzanthracene, and 10-methyl-1,2-benzanthracene. In addition, the choleic acids of related noncarcinogenic compounds of phenanthrene, 1,2-benzanthracene, and acenaphthene were tested.[1]

To determine the effect of these compounds several related groups of experiments were designed. In one group the effect upon fertilization and cleavage was tested with *Arbacia* eggs. In the next group of experiments permeability to water was studied using the marine eggs as the test cells; in another group, a variety of solutes having different penetrating properties were investigated using erythrocytes; and in a fourth we tested with erythrocytes the possibility that the compounds might alter the cell membrane in such a way as to change the lytic action of butyl alcohol.

Experiments with Egg Cells

One of the chief advantages of these cells is that they lend themselves not only to quantitative measurements of permeability, but with them it is also possible to determine whether the agents used have altered fertilization and cell cleavage, and whether they have produced injury or death (2). If the experimental conditions have not affected the cells adversely, the addition of sperm will lead to well-defined changes of cell surface, such as development of a fertilization membrane, and subsequently to orderly cleavage. If, on the other hand, the cells have been affected, then surface changes or cleavage may take place in atypical manner or not at all, and, if the degree of alteration is sufficiently large permeability is changed as well.

The experiments were carried out as follows:

Sea urchin eggs were placed in saturated solutions of the choleic acid compounds of the hydrocarbons in

* This investigation was aided by a grant from The International Cancer Research Foundation.

[1] These compounds were prepared in Professor Louis Fieser's laboratory at Harvard University. We are indebted to Drs. G. H. A. Clowes and M. E. Krahl of the Lilly Research Laboratory, who, with Prof. Fieser's consent, generously shared with us samples of these compounds.

sea water.[2] After an exposure of 2 hours sperm was added and the effects of insemination were observed. The results of a representative experiment are shown in Table I. It will be noted that all of the 3 carcinogenic compounds have retarded cell division and induced atypical cleavage, wheras the related noncarcinogens did not affect the cells adversely. In other experiments of this kind similar results were obtained. Thus, under the stated conditions of concentration and exposure, the carcinogenic compounds were found to produce clearly demonstrable effects upon the cleavage process of the egg cells.

TABLE I: EFFECT OF CHOLEIC ACIDS OF CARCINOGENIC AND RELATED NONCARCINOGENIC HYDROCARBONS UPON CELL CLEAVAGE *

Experiments	Results
Control in ordinary sea water	Over 90 per cent of eggs have undergone normal cleavage and are in 4 cell stage.
Choleic acids of:	
10-Methylbenzanthrene	All cells have fertilization membrane, but over 50 per cent have failed to undergo cleavage; the remainder are in 2 or 4 cell stage with many very atypical forms.
20-Methylcholanthrene	50 per cent in 2 cell stage, remainder in 4 cell stage. Many atypical forms.
1,2,5,6-Dibenzanthracene	Approximately one-third of the cells have failed to cleave and some of these are cytolyzed. Remainder in atypical 2 and 4 cell stage.
Phenanthrene	Over 90 per cent in normal 4 cell stage.
Acenaphthene	Over 90 per cent in normal 4 cell stage.
1,2-Benzanthracene	Over 90 per cent in normal 4 cell stage.

* Sea-urchin eggs were exposed for 2 hours to saturated solutions of the compounds in sea water; sperm were then added. The results refer to the condition of the eggs 100 minutes after insemination (temperature was 22° C.).

We now come to experiments on permeability of these cells to water. In these experiments the time of exposure to the several compounds was lengthened to from 3 to 4 hours, in order to accentuate their effects.[3] After exposure the cells were placed in 50 or 60 per cent sea water.[4]

In these hypotonic solutions water entered the cells under the driving force of osmotic pressure, and the cells swelled. The course of swelling was followed by a diffraction method which permits accurate measurements of cell size at intervals of a few seconds (3). From such experiments, the permeability of the cells to water can readily be calculated from the equation

$$K = \frac{dV}{dt} / S \cdot \Delta P$$

where $\frac{dV}{dt}$ is rate of change of cell volume due to penetration of water, S cell surface, Δ P the difference in osmotic pressure between cell interior and medium, and K expresses the permeability of the cell to water (4). The numerical value of permeability for the cells of the two species used is, on the average, 0.1 for the *Arbacia* egg, and 0.4 for the *Chaetopterus* egg, at 22° C.; these figures signify the number of cubic micra of water which pass per minute through each square micron of cell surface, under the driving force of 1 atmosphere of pressure. It will not be necessary to discuss the computation of permeability further, as the results of the experiments may clearly be shown graphically.

In Fig. 1 observed volumes of cells, measured during their course of swelling in hypotonic sea water, are plotted against corresponding times; the smooth curves represent the course of swelling of control cells. It is seen that for both kinds of cells, *Arbacia* and *Chaetopterus,* the volumes of the exposed cells are closely scattered about the curve of the control cells.

From these and many similar experiments it was found that these carcinogenic hydrocarbons did not alter to a measurable degree the permeability of these particular cells to water. This result was unexpected, since exposure to these compounds led to decided disturbances of cell cleavage—an effect which is usually associated with change in permeability (2).

EXPERIMENTS WITH ERYTHROCYTES

Mammalian erythrocytes have peculiar advantages as experimental material for the study of cell permeability in that their physiological properties are remarkably constant and their structure is very simple; on account of these characteristics no other type of animal cell has been as extensively employed for such investigations (1). In the present experiments erythrocytes of two species, mouse and beef, were used to test the effect of the carcinogens upon penetration of various kinds of solutes. The cells were washed in isotonic saline and then suspended in saturated solutions of the choleic acid compounds of the hydrocarbons (dissolved in isotonic saline). The suspensions were mechanically shaken in the absence of

[2] These compounds are said to be photosensitive, hence solutions were prepared in dim light, and the cells were kept in the dark during exposure. All solutions were slightly milky in appearance and contained undissolved residue.

[3] In the 20-methylcholanthrene solutions the cells tended to stick to the glass container; otherwise exposure to the several compounds did not induce visible changes.

[4] By 60 per cent sea water is meant a solution consisting of 60 parts of sea water and 40 parts of distilled water.

light for periods of 6 hours. Control cells, not exposed to the hydrocarbons, received similar treatment.

The permeability of exposed and control erythrocytes was tested with respect to a variety of solutes having widely different penetrating properties. One group—ethylene glycol, glycerol, erythritol, and thiourea—is generally thought to penetrate in molecular form through aqueous channels in the cell membrane; another group—Cl and SO_4 salts—penetrates in ionic form. A third group—NH_4 acetate and NH_4 butyrate—probably penetrates by virtue of their solubility in the lipids of the cell membrane.

A photoelectric method was used to determine the rate of penetration (6). This method is based upon the principle that when a compound penetrates into erythrocytes the cells swell osmotically to a point where hemoglobin diffuses out; the rate at which solute. In Table II are given results of typical experiments from which it is concluded that the permeability of mouse erythrocytes for glycerol and erythritol is altered by none of the three carcinogens or the related noncarcinogens.

TABLE II: EFFECT OF CHOLEIC ACIDS OF CARCINOGENIC HYDROCARBONS UPON PERMEABILITY OF MOUSE ERYTHROCYTES TO GLYCEROL AND TO ERYTHRITOL

Mouse erythrocytes exposed to choleic acids of:	Time for 50 per cent hemolysis due to penetration of: Glycerol	Erythritol
20-Methylcholanthrene	25.5 sec.	7 min. 10 sec.
1,2,5,6-Dibenzanthracene	24.8 sec.	6 min. 50 sec.
10-Methyl-1,2-Benzanthracene	25.0 sec.	7 min.
Phenanthrene	25.0 sec.	6 min. 58 sec.
1,2-Benzanthracene	22.5 sec.	7 min. 4 sec.
Acenaphthene	23.5 sec.	6 min. 58 sec.
Control	24.5 sec.	7 min.

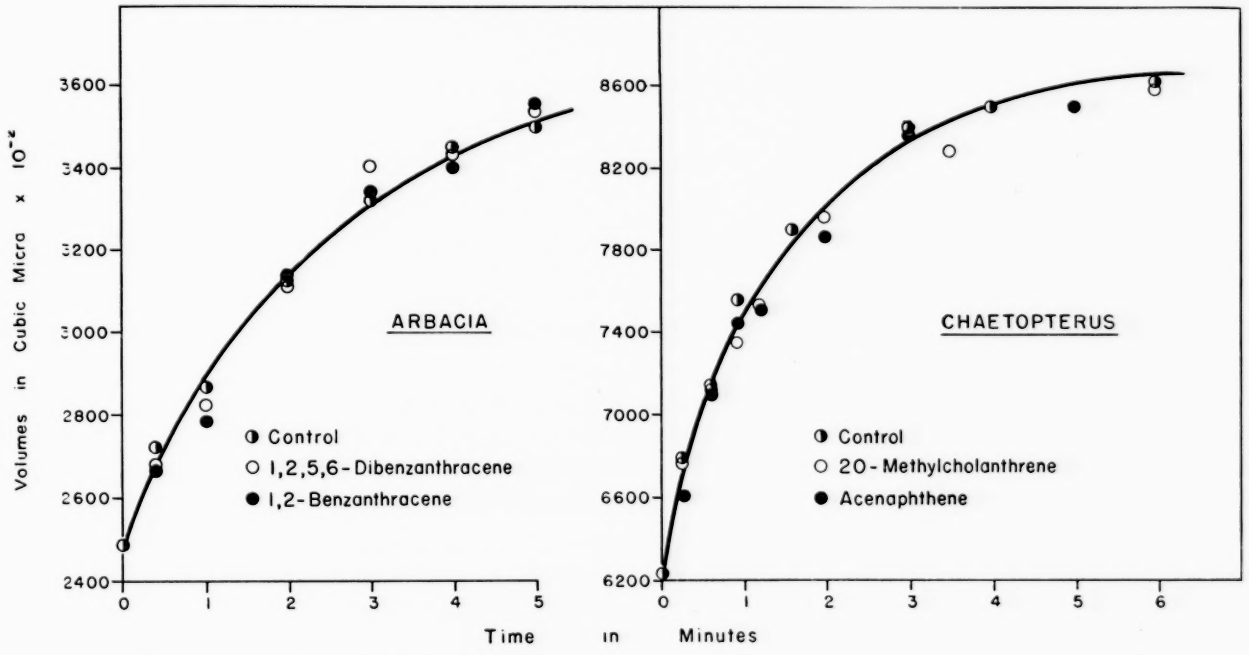


FIG. 1.—Two representative experiments which illustrate the course of swelling in hypotonic sea water of *Arbacia* and *Chaetopterus* egg cells after exposure to carcinogenic and noncarcinogenic choleic acid compounds. The volumes of the exposed cells are closely scattered around the curves of the control cells.

this occurs may be determined with great precision. The results of the experiments are obtained as photographic (galvanometric) records. A typical record is shown in Fig. 2; in this experiment glycerol was chosen as the penetrating solute. It will be noted that the cells exposed to choleic acids of two of the carcinogens, 20-methylcholanthrene and 1,2,5,6-dibenzanthracene, give the same kind of curve as do control cells or cells exposed to a noncarcinogenic compound, phenanthrene.

For quantitative comparison of effects, the time for half completion of osmotic hemolysis serves as an excellent criterion for the rate of penetration of a

Similar results have been obtained from experiments with both mouse and beef erythrocytes when their permeability was tested for thiourea, ethylene glycol, Cl ions, SO_4 ions, and ammonium salts of acetic and butyric acids. In no case was the permeability of the cells exposed to either the carcinogenic or the noncarcinogenic agents significantly changed.

In a final group of experiments we measured the time required for butyl alcohol to hemolyze erythrocytes previously exposed to the several carcinogenic and noncarcinogenic compounds. The method employed was similar to that used in the preceding group of experiments. The results showed that these

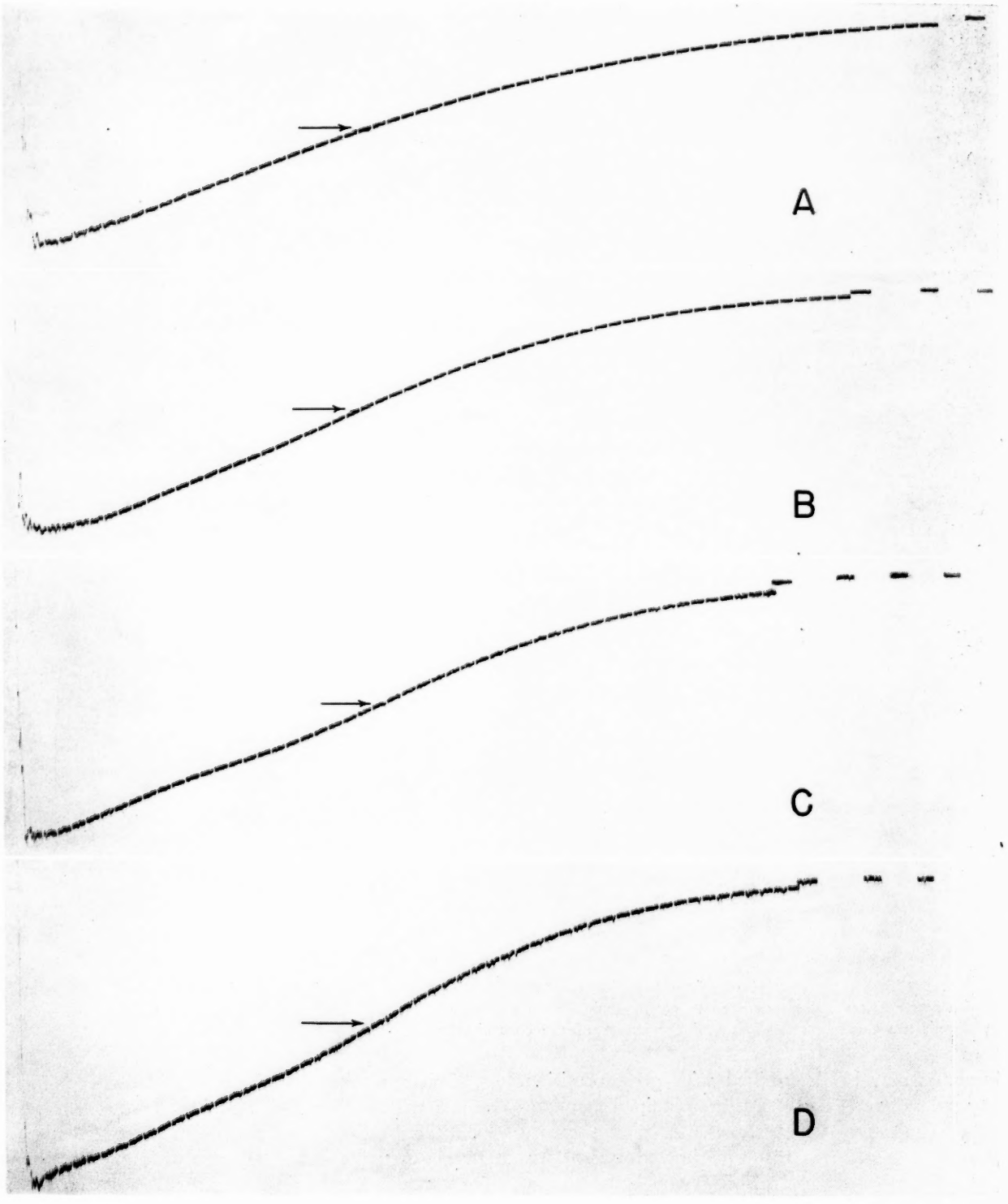


FIG. 2.—Photographic records of the penetration of glycerol into mouse erythrocytes after exposure to choleic acids of carcinogenic and noncarcinogenic compounds. Quick downstroke at left indicates mixing of the cells with an isosmotic (0.3 M) solution of glycerol. Slow attainment of a plateau indicates osmotic hemolysis accompanying the penetration of glycerol. Arrow denotes ½ completion of this penetration. Time is marked in one second intervals by the vertical clear spaces on the continuous record, the final portions of the photographs mark intervals of 15 seconds. A, swelling of unexposed control cells; B and C, swelling after exposure to 20-methylcholanthrene and 1,2,5,6-dibenzanthracene, respectively; D, swelling after exposure to phenanthrene.

compounds did not alter the resistance of the cell membrane to the lytic destructive action of butyl alcohol.

Summary

Several types of cells which permit quantitative study of permeability (egg cells of *Arbacia* and of *Chaetopterus,* erythrocytes of mouse and beef) were exposed to choleic acid compounds of carcinogenic and related noncarcinogenic hydrocarbons; permeability of the cells to water and to a variety of solutes was then measured. The carcinogens chosen for these experiments were choleic acid compounds of 20-methylcholanthrene, 1,2,5,6-dibenzanthracene, 10 methyl-1,2-benzanthracene; the related noncarcinogens were similar compounds of phenanthrene, 1,2-benzanthracene, and acenaphthene.

The results of the determinations showed that neither the cells exposed to the choleic acids of these carcinogens nor those exposed to related noncarcinogens differed in their permeability from the control unexposed cells. This was true for both marine eggs and erythrocytes.

Under similar experimental conditions the carcinogens did, however, induce decided disturbances of cell cleavage (using *Arbacia* eggs as the test objects).

References

1. Jacobs, M. H. Osmotic Properties of the Erythrocyte. Biol. Bull., **62**:178-194. 1932.
2. Lucké, B. and M. McCutcheon. The Effect of Injury on Cellular Permeability to Water. Arch. Path., **10**:662-670. 1930.
3. Lucké, B., M. G. Larrabee, and H. K. Hartline. Studies on Osmotic Equilibrium and on the Kinetics of Osmosis in Living Cells by a Diffraction Method. J. Gen. Physiol., **19**:1-17. 1935.
4. Lucké, B., H. K. Hartline, and R. A. Ricca. Comparative Permeability to Water and to Certain Solutes of the Egg Cells on Three Marine Invertebrates, Arbacia, Cumingia and Chaetopterus. J. Cell. & Comp. Physiol., **14**:237-252. 1939.
5. Magath, M. A. Über Beziehungen zwischen Permeabilität und Stoffwechseltypus der Tumorzelle. Ztschr. f. Krebsforsch., **36**:205-216. 1932.
6. Parpart, A. K. The Permeability of the Mammalian Erythrocyte to Deuterium Oxide (Heavy Water). J. Cell & Comp. Physiol., **7**:153-162. 1935.
7. Rohdenburg, G. L., and A. Bernhard. Einige physikalisch-chemische Ausblicke auf das Problem der malignen Zelle. Ztschr. f. Krebsforsch., **28**:301-310. 1929.
8. Schmidtmann, M. and K. Matthes. Untersuchungen über die Reaktion und Permeabilität von Zellen des entzündeten und Geschwulstgewebes. Ztschr. f. die ges. exper. Med., **57**:127-144. 1927.

Genetic Resistance to a Transmissible Sarcoma in the Fowl

Randall K. Cole, Ph.D.

(From the Department of Poultry Husbandry, Cornell University, Ithaca, New York)

(Received for publication July 10, 1941)

The problem of the prevention or control of neoplastic diseases is not limited to the human race for these diseases constitute one of the major causes for adult mortality in the domestic fowl. The more common neoplasms of the fowl differ somewhat from those appearing in other species in that the etiological agents in many instances appear to be virus-like in nature and also in that the sarcomas far outnumber the carcinomas. The neoplastic conditions are in evidence in the early life period of the birds (6 to 18 months) and at this age nearly all of the tumors belong to the sarcoma group, primarily those involving the lymphoid type of cell. Adenocarcinomas involving the ovary and peritoneum are not infrequent in the older birds.

The reduction of adult flock mortality through suitable breeding methods (5, 8, 2, 4) strongly suggests an avenue of approach to the perplexing problem of the control of neoplastic diseases affecting the fowl. That the genetic constitution of the individual can markedly affect the probability of its developing a spontaneous neoplasm is well known to the mouse breeder. Although one might reason from one species to the other, it would be amiss to draw similar conclusions without tangible evidence.

If genetic resistance to neoplasms does exist in the fowl—and this would have to be a major premise for those far-reaching programs of breeding for disease resistance now in progress—then it should be possible to demonstrate such resistance through suitable experimental procedures. The experiments reported in this paper were organized for the purpose of determining the extent to which resistance to a transmissible sarcoma could be increased by genetic selection.

Plan of the Experiment

Choice of neoplasm.—A transmissible sarcoma, similar to the Rous sarcoma, was selected because of its easy transmission, its short incubation period, and its viability when preserved as dehydrated tumor tissue. This transmissible sarcoma was obtained from Dr. Edwin Jungherr of the Storrs Agricultural Experiment Station who has reported some results (6) that he obtained with this tumor.

Histologically, the Jungherr sarcoma consists primarily of spindle cells, a varying amount of mucoid secretion, a minimum vascular supply, and occasional lymphocytic accumulations which are usually associated with necrotic areas in the tumor. Depending upon the location of the tumor growth, the amount of mucoid secretion varies widely. When the tumor is compressed by the surrounding tissues, following intramuscular injection, for example, the mucoid secretion is at its minimum and the tumor itself is relatively firm and compact. Following intraperitoneal injection, the accumulating mucoid exudate often causes marked distention of the abdomen and interference with respiration. In this situation, the tumor tissue is quite soft and of a gelatinous consistency.

Method of testing for resistance to the Jungherr sarcoma.—All chicks used were from fully pedigreed stock and were inoculated with aqueous suspensions of fresh tumor tissue at the age of 7 to 18 days. The inoculum consisted of an approximately 3 per cent suspension of fresh tumor tissue in distilled water. In the early tests, the chicks received 0.2 to 0.5 cc. of the suspension intraperitoneally. In order to facilitate easy determination of susceptibility, the site of inoculation was changed to the muscles of the wing (fore-arm). Each chick then received 0.2 cc. of the inoculum intramuscularly in each wing. Tumor development could, at this site, be easily determined by palpation.

All chicks were classified for the development of tumor at 2, 4, and 6 weeks following inoculation. Except in the original unselected population where intraperitoneal inoculation was used, chicks showing tumor development at both sites of inoculation were removed from the experiment when first detected and were recorded as susceptible. Chicks which showed no development of the tumor at the end of the 6-week test period were recorded as resistant. Those showing tumor growth in one wing only were kept until the end of the test period, when they were recorded as susceptible. Chicks which died or were in poor physi-

cal condition at the end of the 6-week test period, unless showing development of the tumor, have been eliminated from the data.

Foundation stock.—The breeding stock in the disease resistant line of Single Comb White Leghorns at the Cornell Agricultural Experiment Station was used in 1936 and 1937 to produce the original experimental chicks. Since these original tests, which included the progenies of 26 dams and 4 sires, all the breeding stock used or chicks tested have been within the groups selected in 1936 and 1937. At that time, families showing a high percentage of individuals susceptible to the Jungherr sarcoma were used to establish the susceptible line. Likewise, families showing a small percentage of susceptible individuals became the foundation for the resistant line. There have been a few cases wherein it was deemed desirable to shift birds from one line to another on the basis of their progenies' or sibs' reaction to the tumor inoculation. In such cases, the results obtained from the testing of the chick families will be summarized in that generation for which they were selected. The parents of these families, if transferred to the opposite line, are then considered, in future breeding tests, on the same bases as the original unselected breeders, and the results obtained by the inoculation of their immediate progenies are also summarized in the unselected population group. Thus, the original unselected population consisted of 664 chicks, representing the progenies of 43 dams and 7 sires. Untested full sibs of many of these chicks were available among the pullet and cockerel population of the Agricultural Experiment Station. Such sibs, from the selected families, were used to produce the first selected generations in the susceptible and resistant lines.

Method of breeding.—The first selected generations were obtained by brother × sister or parent × progeny matings. In the later generations, the inbreeding has been much less intense although the breeding has been done within relatively small closed populations.

The principle of the progeny test has been used almost entirely in the breeding plan. From each population of pullet breeders to be tested by the tumor inoculation of their immediate progenies, the more vigorous birds were chosen. In general, the full populations of potential breeders in each line were observed for a period of time prior to test so that some selection with respect to egg size, intensity of production and viability could be made. These economic characters are of much importance to the maintenance of the selected lines.

Equal numbers of the physically best birds in each line were mated to selected males from their respective lines. Except in the testing of the original unselected breeders, the procedures of testing were carried out in the late fall and early winter months. On the bases of the results obtained at this time, certain of the better matings, from the standpoint of the reactions of the chick families to the tumor inoculation, were continued through the following breeding season so that the next generation of breeders could be produced. The breeders have been selected, therefore, on the bases of the reaction of that proportion of their sibship which was actually inoculated with tumor rather than their own individual reaction to the test.

TABLE I: INCIDENCE OF SUSCEPTIBILITY TO THE JUNGHERR SARCOMA

Source of chicks	Chicks tested	Susceptible			Sex difference	
		Total *	Males	Females	♀-♂	Significance of difference
	Number	Per cent	Per cent	Per cent	Per cent	P-value
Susceptible line	1,558	68.5	64.9	69.9	5.0	> 0.02
Resistant line	730	43.4	40.9	42.9	2.0	< 0.70
Unselected controls	664	70.6	69.7	70.1	0.04	< 0.80
Actual total	2,676	64.2	62.0	64.7	2.7	> 0.10

* Includes 81 chicks (all susceptible) for which sex was not recorded.

RESULTS OF SELECTION FOR RESISTANCE TO THE SARCOMA

Differentiation of lines.—An actual total of 2676 pedigreed chicks has been tested, of which 1719 or 64.2 per cent were susceptible to the Jungherr sarcoma. Since certain of the chick families are included under more than one classification, depending upon their pedigree and/or the future use to which the breeder has been assigned, the sum of the subtotals of susceptible, resistant and control chicks will exceed the actual total. The results of all tests are shown in Table I, from which it can be noted that selection for susceptibility has apparently not been very effective while selection for resistance has resulted in a significant increase in resistance among chicks of the resistant line.

The effects of genetic selection for resistance or susceptibility to the Jungherr sarcoma are readily shown in Fig. 1, in which the data are grouped according to the number of generations of selection

in the ancestry of the chicks tested. Since the sire and dam frequently did not possess the same number of generations of selection, the progeny therefore might actually represent only 2½ or 2⅞ generations of selection. For the convenience of presenting the data, families possessing more than one generation, up to and including two generations of selection, are considered as representing two generations of selection.

Since the chicks belonging to the third generation in the resistant line were not tested at the same time as those in the third generation in the susceptible line, the data are summarized in Table II on the basis of the year of test. The above mentioned differences were brought about because the foundation stock for the resistant line was not located in the same year as that for the susceptible line. In Table II, the chicks in the resistant line for 1939-40 may belong in the first, second, or third generation, and likewise the susceptible line chicks may belong in the second, third, or fourth generation.

Table II: Yearly Progress in Differentiating the Lines Resistant and Susceptible to the Jungherr Sarcoma

Line	Year of test: 1937-1938 Chicks Number	1937-1938 Susceptible Per cent	1938-1939 Chicks Number	1938-1939 Susceptible Per cent	1939-1940 Chicks Number	1939-1940 Susceptible Per cent
Susceptible	368	77.4	388	61.8	502	71.9
Resistant	157	65.6	329	39.8	189	30.2
Difference		11.8		22.0		41.7
P-value *		< 0.01		< 0.001		< 0.001

* Based upon the Chi-square test in the four-fold contingency table, using the number of chicks. A P-value of less than 0.01 indicates that the difference is highly significant.

It is evident from Fig. 1 and Table II that, with each succeeding generation or year, the two lines are becoming more divergent with respect to susceptibility to the Jungherr sarcoma. This in itself indicates the genetic nature of the resistance and susceptibility to the tumor and the effectiveness of the breeding procedure to intensify or increase the desired character in the population.

Differences within lines.—As the experimental work has progressed, it has become evident that one would observe the expected rather marked differences in the breeding ability of birds with similar ancestry. It has therefore been necessary to test several members of each family of potential breeders before the final selections for the breeding pens are made in order to maintain steady improvement in either line. This is the method of operating the progeny test system, for it is only by the reaction of the progeny (in this case, the ability to produce highly resistant or susceptible chicks) that we can evaluate the breeder.

Influence of Sex on Resistance

Although in some instances (3, 7), the sex or the sex hormones play an important part in resistance to disease, there is apparently no relationship between sex and resistance to the Jungherr sarcoma. Table I includes the data which indicate the lack of any marked relationship between sex and resistance to the Jungherr sarcoma among the White Leghorn chicks tested in the course of the present investigations.

Rate of Growth of the Tumor as a Measure of Resistance

The rather frequent positive correlation of high susceptibility with early mean age at death or severity of lesions was found to be present in the current experiments. Following intraperitoneal inoculation the tumors developed very rapidly in susceptible chicks and led to early death. The 86 progeny of the 2 most susceptible sires of 4 tested not only had a high final proportion of susceptible individuals (77 per cent at 49 days) but the first deaths occurred 13 and 14 days following inoculation. The two most resistant sires (43 chicks) with a mortality of 37 per cent at 49 days did not lose their first progenies until 23 and 25 days following inoculation. Both of the susceptible sires had lost 20 per cent of their progenies before the first death occurred in progeny of the two more resistant sires.

In one series with intramuscular (wings) inoculation, 3 susceptible line females produced 94 chicks, of which 83 were growing the tumor at both inoculation sites at the end of 2 weeks. Four others had palpable tumor growth in one wing. At the end of 4 weeks, all 94 chicks were growing tumor in both wings. Two resistant line females produced 74 chicks in this series, of which only 5 were growing the tumor in both wings, and 3 in one wing, at the end of 2 weeks. At 4 weeks, there were 7 and 3 respectively, and at the end of the test period only 8 chicks had tumor growth in both wings, while another 8 chicks had tumor in one wing only. Thus, the resistant families exhibited their resistance not only by the relatively low proportion of susceptible individuals (22 per cent), but also by the slowness with which the tumors developed and the fact that one-half of those susceptible were able to limit tumor growth to one of the two inoculation sites.

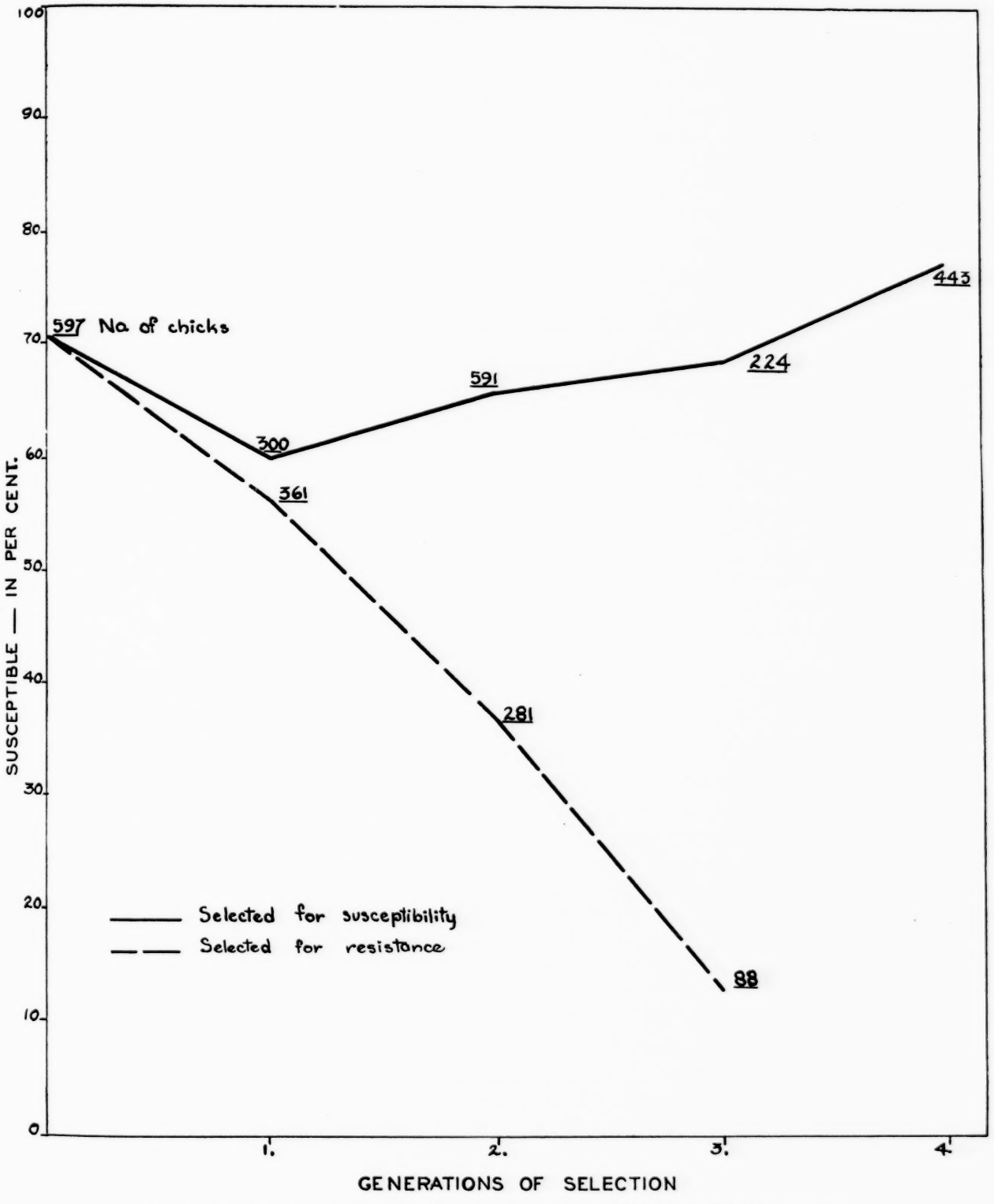


FIG. 1.—Results of genetic selection for resistance or susceptibility to the Jungherr sarcoma.

The Effect of Age on Resistance

In order to check the possibilities of the phenomenon of age resistance (9, 1) with respect to the Jungherr sarcoma, the results of a rather limited series of tests are available for analysis.

In the winter of 1937-38, the chicks were of two general age groups at the time of inoculation (Table III).

Table III: The Effect of Age on Resistance to the Jungherr Sarcoma

Age at inoculation	Chicks inoculated	Resistant
Days	Number	Per cent
7-11	250	30.0
14-18	275	22.9
	Difference	7.1

The slight lowering of resistance among the older chicks is not significant, the P value being greater than 0.05. One possible explanation for the slightly higher incidence among the older group is that the older and larger chicks were able to retain the full amount of inoculum within the muscles of the wing. In some instances, small portions of the inoculum were expressed by muscular contraction in the younger chicks.

Resistance to the Jungherr Sarcoma as a Measure of Resistance to Diseases in General

One of the principal ideas back of these experiments involved the possibility that resistance to the Jungherr sarcoma might indicate to some extent a general resistance to disease and in particular to neoplastic diseases.

Toxic wheat-germ meal.—The excessive mortality of approximately 50 per cent by 8 weeks of age in the chicks hatched during the winter of 1938-39 offered an opportunity to determine if the resistant and susceptible lines differed with respect to their reaction to the apparently toxic agent in the wheat-germ meal which was considered responsible for the mortality. The data are presented in Table IV.

Table IV: Resistance to the Jungherr Sarcoma versus Resistance to Toxic Wheat-germ Meal

	Chicks hatched	Chicks to complete test	Susceptible to tumor	Mortality to 8 weeks
	Number	Number	Per cent *	Per cent
Resistant line	619	329	39.8	46.9
Susceptible line	824	388	61.8	52.9
		Difference	22.0	7.0

* Of those to complete test.

The differences between the two lines in susceptibility to the tumor (22 per cent) is highly significant. The chicks resistant to the tumor were also somewhat more resistant to the toxic wheat-germ meal although the observed difference is not highly significant, P lying between 0.02 and 0.01.

Diseases in general.—A comparison of the viability of pullets between the ages of 160 and 500 days from the two selected lines is presented in Table V.

It is apparent from the data presented in Table V that the two lines which differ rather markedly with respect to resistance to the Jungherr sarcoma do not at the same time differ in viability during the age period of 160 to 500 days nor in the incidence of spontaneous neoplasms (mostly lymphomatosis). The higher mortality, both total and neoplastic, for the 1940-41 pullets in the resistant line is based upon a portion of the test period. The relative standing of the two lines may change much in the next 90 days and therefore interpretation of the 1940-41 data must await the completion of the test.

Further evidence that resistance to the Jungherr sarcoma does not ensure resistance to diseases in general is found in the results of a study (Table VI) of the progenies of the original unselected breeders. If that portion of the progeny inoculated with the sarcoma was highly susceptible, there was a general tendency that the uninoculated control female sibs would be more viable, during both the rearing and adult periods, than would the control sibs from families which were more resistant to the Jungherr sarcoma.

The trend suggests that resistance to the Jungherr sarcoma is not indicative of resistance to the common causes of mortality. The 9 families which showed a relatively high degree of resistance to the Jungherr sarcoma (44 per cent) were on the contrary comparatively susceptible (16.5 per cent) to the causes of mortality during the rearing period (1 to 159 days) and also (59.5 per cent) during the period of 160 to 500 days of age.

Discussion

The fact that it has been possible to differentiate two lines of White Leghorns with respect to resistance to a transmissible tumor would indicate that in the fowl the development of neoplasms is dependent to a certain extent upon the genetic constitution of the individual bird. By using a transmissible sarcoma, it has been possible to minimize the influence of nongenetic factors.

The transmission of the tumor consisted essentially of grafting neoplastic tissue. The genetic constitution of the host measured is really that which permits

the development of the transplanted tumor cells. This genetic constitution may be very similar to, or may differ widely from, that which permits the original development of the tumor cells by the action of the virus. Since using the virus in the form of desiccated tumor results in fewer takes and a much longer and varied incubation period, it was necessary to rely on fresh tumor tissue suspensions for inoculum.

An attempt was made to standardize the source of the inoculum by using chicks unrelated to either selected line. When this was not feasible tumor tissue material was taken from one chick in each of the two selected lines. In this manner, some consideration was given to the well-known fact that tissue transplantation is more successful between the same, or closely related, individuals than it is apt to be between nonrelated individuals.

The relatively high degree of susceptibility among the control chicks (70.6 per cent) is due primarily to the fact that 3 of the 4 original unselected sires produced progenies which were quite susceptible to the tumor inoculation. Within the susceptible line, however, there was not only a tendency for increasing susceptibility, but also for a less variable reaction on the part of the different progenies. The 11 F_1 daughters of an original breeder (B1598) produced 216 chicks, of which 67.1 per cent were susceptible. The susceptibilities among the progenies of the 11 daughters varied from 17 to 93 per cent. In the F_4 generation of this line, the 7 female breeders produced 134 chicks, of which 91 per cent were susceptible, while the range was from 64 to 100 per cent. Thus, while there has been some increase in susceptibility within the susceptible line, there has also been a marked tendency for greater uniformity. Within the resistant line, the marked improvement is of itself adequate proof of the effectiveness of genetic selection.

The apparent lack of any relationship between resistance to the sarcoma and resistance to other causes of disease, unless it be reciprocal, merely indicates that the genes required for resistance to this tumor are independent of those involved in resistance to other etiological agents. The differences in the degree of inbreeding within the lines have varied so that it is not advisable to place much emphasis upon any differences between the lines with respect to viability in general. In the data from the original unselected breeders, where this criticism is not valid, there is not a sufficient spread in the susceptibility to the tumor to permit the division of the families into several subgroups for the purpose of comparing general viability. Therefore, in this connection it is possible to say only that resistance to the Jungherr sarcoma does not necessarily indicate resistance to disease in general.

TABLE V: MORTALITY IN THE PERIOD OF 160-500 DAYS OF AGE AMONG PULLETS FROM THE LINES RESISTANT AND SUSCEPTIBLE TO THE JUNGHERR SARCOMA

	Resistant line			Susceptible line		
		Mortality—160-500 days			Mortality—160-500 days	
Year	Pullets Number	Total Per cent	Neoplastic Per cent	Pullets Number	Total Per cent	Neoplastic Per cent
1938-39	79	54	19	114	52	13
1939-40	41	51	15	25 *	64	20
1940-41 †	43	56	28	90	40	17

* Many additional birds marketed in January and February, 1940.
† To June 1, 1941, only.

TABLE VI: RELATIONSHIP OF RESISTANCE TO THE JUNGHERR SARCOMA TO RESISTANCE TO OTHER CAUSES OF MORTALITY

Relative degree of resistance	Jungherr sarcoma			Range * mortality		Adult † mortality	
	Families Number	Chicks Number	Resistant Per cent	Pullets Number	Dead Per cent	Pullets Number	Dead Per cent
High	9	134	44.0	139	16.5	116	59.5
Medium	8	148	10.7	142	4.9	135	37.8
Low	9	161	2.5	181	7.7	167	43.7

* To 159 days of age.
† 160 to 500 days of age.

SUMMARY AND CONCLUSIONS

Genetic resistance to a transmissible sarcoma has been demonstrated with the fowl. By using the progeny test as a method of selecting breeders, it has been possible to differentiate two lines of White Leghorns, one characterized by a relatively high degree of resistance (resistant line) and the other by a relatively low degree of resistance (susceptible line) to the Jungherr sarcoma. The study has involved 2676 pedigreed chicks, of which 64.2 per cent were susceptible to the tumor inoculation. After 4 genera-

tions of selection, 77.2 per cent of the chicks in the susceptible line developed the tumor following inoculation; while after 3 generations of selection, 12.5 per cent of the chicks tested in the resistant line were susceptible. Although susceptibility has not been increased very much over that for nonselected control chicks, there has been a very definite reduction of variability within the susceptible line. The difference in resistance between the two selected lines has increased with each succeeding generation. Susceptibility to this transmissible tumor is apparently not influenced by sex or by the age of the host. A high degree of susceptibility is indicated not only by the large proportion of the family which develops the tumor, but also by the very short incubation period.

From the evidence now available, it appears that resistance to the Jungherr sarcoma is not indicative of resistance to the common causes of poultry mortality.

REFERENCES

1. Ackert, J. E., D. A. Porter, and T. D. Beach. Age Resistance of Chickens to the Nematode Ascaridia Lineata (Schneider). J. Parasitol., **21**:205-213. 1935.
2. Bearse, G. E., C. F. McClary, and M. W. Miller. The Results of Eight Years' Selection for Disease Resistance and Susceptibility in White Leghorns. Poultry Sc., **18**: 400-401. 1939. (abstract)
3. Campbell, Dan H. The Effect of Sex Hormones on the Normal Resistance of Rats to Cysticercus Crassicollis. Science, **89**:415-416. 1939.
4. Gildow, E. M., J. K. Williams, and C. E. Lampman. The Transmission of and Resistance to Fowl Paralysis (Lymphomatosis). Idaho Agric. Exp. Sta. Bull., **235**:2-22. 1940.
5. Hutt, F. B., J. H. Bruckner, and R. K. Cole. Reduction of Mortality in Fowls by Breeding. Proc. 7th World's Poultry Congress, 49-51. 1939.
6. Jungherr, Erwin. Studies on Fowl Paralysis. 2. Transmission Experiments. Storrs (Conn.) Agric. Exp. Sta. Bull., **218**:5-47. 1937.
7. Madsen, D. E. An Erysipelas Outbreak in Turkeys. J. Am. Vet. Med. Assn., **91**:206-208. 1937.
8. Marble, D. R. Breeding Poultry for Viability. Pennsylvania Agric. Exp. Sta. Bull., **377**:1-38. 1939.
9. Rettger, L. F., W. F. Kirkpatrick, and F. H. Stoneburn. Bacillary White Diarrhea of Chicks (Third Report). Storrs (Conn.) Agric. Exp. Sta. Bull., **74**:153-185. 1912.
10. Taylor, L. W., and I. M. Lerner. Breeding for Egg Production. California Agric. Exp. Sta. Bull., **626**:3-48 1938.

Rapid Growth of a Bronchiogenic Carcinoma

M. S. Eveleth, M.D., and Norman C. Wetzel, M.D.

(From the Institute of Pathology and the Department of Pediatrics, Western Reserve University, and University Hospitals of Cleveland, Cleveland, Ohio)

(Received for publication June 27, 1941)

Bohrod (1) has reported a pleural mesothelioma with an unusually rapid growth rate, having an average daily rate of growth of 27.6 gm. This estimate of growth rate of an internal tumor was possible because a known amount of fluid was removed from the chest and this space was filled with tumor at the time of autopsy.

The case we are reporting in this communication offered an exceptional opportunity to observe the rate of "free" growth of a human carcinoma. The removal of a lung left an open pleural sac into which there was neoplastic proliferation from an exceedingly small residual mass of bronchiogenic carcinoma, proceeding without any restrictive influence of immediately neighboring tissue.

Case Report

A 39-year-old white man entered the hospital for the first time on November 14, 1940, complaining of coughing up blood-streaked sputum and shortness of breath for 11 months. Two months before this admission he had a spontaneous pneumothorax proven by fluoroscopy.

On this admission no positive physical findings were noted over the left thorax. Bronchoscopy revealed no lesions, but bronchograms taken after injection of lipiodol showed downward displacement of the bronchi of the left upper lobe. Roentgenograms of this lung showed increased air content of the upper part of the left upper lobe. These findings were interpreted as being due to a large emphysematous bleb or a congenital cyst in this region. There was also an oval shadow of increased density at the upper pole of the left hilum. The patient was discharged after 9 days in the hospital because he felt better and the lesion was thought to be benign.

Subsequent to his discharge, he went back to work but became more short of breath, weaker, and continued to have hemoptysis. He was readmitted about one month later, December 26, 1940. On this admission he had a temperature of 38° C. and there were signs of a left pneumothorax. At this time the provisional diagnosis was cystic disease of the left upper lobe with secondary infection and it was thought advisable to perform a lobectomy. On January 6, 1941, at operation, a yellowish gray tumor mass was found in the upper lobe extending down through the interlobar fissure into the lower lobe. Consequently the left lung was completely removed. As the mass was well circumscribed and there were no palpable lymph nodes or pleural metastases noted it was thought that all of the tumor had been removed. Postoperatively he developed a left bronchopleural fistula and empyema which was drained. The patient was discharged, still draining pus from the left pleural cavity, on February 9, 1941.

He re-entered the hospital about 2 weeks later, on February 24, 1941, because he was still draining pus. On this last admission a rib resection was done to provide more adequate drainage. At the time of operation the pleural cavity was seen to be partially filled with tumor tissue.

The lung removed at operation, after injection with formalin, contained a large friable white tumor mass in the upper lobe measuring 8 x 6.5 x 5 cm. and extending down into the lower lobe near the hilum. Microscopically the tumor proved to be carcinoma simplex. Indenting the tumor in the upper lobe near the apex was a large emphysematous cyst measuring 5 x 4 x 3 cm. A calcified nodule was found in the lower lobe near the pleural surface.

The patient became increasingly weaker and died 8 days after this second operation and 58 days after the pneumonectomy.

At autopsy the thorax was symmetrical and there was a recent left thoracotomy with resection of a portion of the 8th rib in the posterior axillary line. Incision through the skin over the left anterior thorax opened into a subcutaneous abscess cavity which extended down between the ribs in this area. The entire left pleural cavity was filled by a mass of pinkish-red soft tumor tissue which was bound down to the ribs and intercostal muscles by firm adhesions. The tumor extended out between the ribs of the left anterior thorax to a subcutaneous position but did not invade the ribs at any point. The tumor mass forced the left leaf of the diaphragm downward so that from the abdominal side this leaf was convex. In the center of the mass there was a large abscess measuring 10 x 5 x 5 cm. which was lined by green necrotic tissue. A bronchopleural fistula opened into this abscess and the abscess communicated through the intercostal spaces with the subcutaneous abscess described above.

The regional lymph nodes, visceral and parietal pericardium over the left atrium, and the head of the pancreas showed gross tumor metastases.

The right lung showed no tumor but did show marked emphysema and in the lower lobe there was bronchopneumonia.

A pure culture of nonhemolytic streptococci was grown from the abscess.

Microscopic sections of the tumor in the lung removed at operation and those from the autopsy material all showed essentially the same appearance. The tumor was composed of sheets of fairly large round or polyhedral cells with no orderly arrangement. The cytoplasm was small in amount, granular, and acidophilic. The nuclei of the cells were oval or round and occupied most of the cell. Some of these nuclei were vesicular and others were hyperchromatic. Numerous mitotic figures were seen, some of which were abnormal. There were numerous blood vessels throughout the tumor and there was a moderate amount of fibrous tissue. Silver stains showed a moderate amount of reticulum. In the veins of the esophagus there were clumps of tumor cells.

The facts at hand are that at the time of pneumonectomy there was no tumor visible to the operator and that 58 days later there was a mass of tumor which

weighed 2,500 gm. There were no exact observations of the tumor in the interval. Thus exact values from day to day cannot be given. Practically, however, two methods of computation can be considered. The average rate of increase in mass is determined simply by dividing 2,500 gm. by 58 days, which gives a daily rate of 43.1 gm., on the assumption that only an immeasurably small mass of tumor remained after operation. This value is certainly too high for the early postoperative days and is too low for a mass which had probably reached at least 800 to 1,000 gm. after approximately 50 days.

In part for these reasons, but also in part because of what is known about cellular reduplication in cultures of various tumor forms, it is more reasonable to assume that the conditions for free or at least comparatively unhampered reduplication were in force throughout the greater part of the 58-day period. No observations or data are at hand to aid in tracing a more exact course, so that the assumption of free reduplication simply implies the following conditions: (a) a small initial seeding (or, in this case, actually a small remnant) of cells capable of reduplication; (b) an adequate source of energy for them, and (c) an environment of high "permittance"; *i.e.,* one with negligible inhibitory effects, whether mechanical, chemical, or metabolic.

TABLE I: SHOWING THE EXPECTED WEIGHT AND CORRESPONDING DAILY RATE OF GAIN IN THE TUMOR MASS UNDER CONDITIONS OF "FREE" AND UNRESTRICTED GROWTH

Time (Postoperative day)	Weight (gm.)	Rate of gain (gm./day)
0	1.0	0.135
10	3.9	0.521
20	14.9	2.0
30	57.4	7.7
40	221.0	29.8
50	854.0	115.1
58	2,500.0	338.0

These conditions lead to utmost simplification of the general equation of growth (4) which reduces, accordingly, to

$$z = Ce^{at} \quad \text{.....I}$$

where z is cell mass in grams, t the time in days, a a constant representing the ratio E/ρ in the paper referred to (4), and C the constant of integration for the initial value of the cell mass at the start, $t=0$, the day of pneumonectomy. Approximate values for z as given by equation I have been computed for 10-day intervals and for the 58th day and are given in Table I, the corresponding exponential curve being shown in Fig. 1.

The values of C and a have been calculated from the fact that the cell mass weighed 2,500 gm. on the 58th postoperative day, the weight on the day of operation being taken, for simplicity, as 1 gm. Thus, $C=1$ and a becomes 0.135. The number of days required to double the mass is $\log_e 2/0.135=5.14$.

On the assumption of free reduplication the tumor mass need, therefore, not have exceeded about 60 gm. at the 30th day, nor 400 gm. as late as the 45th day. The chief gain would have occurred after the 45th day or during the last 10 days to 2 weeks of life.

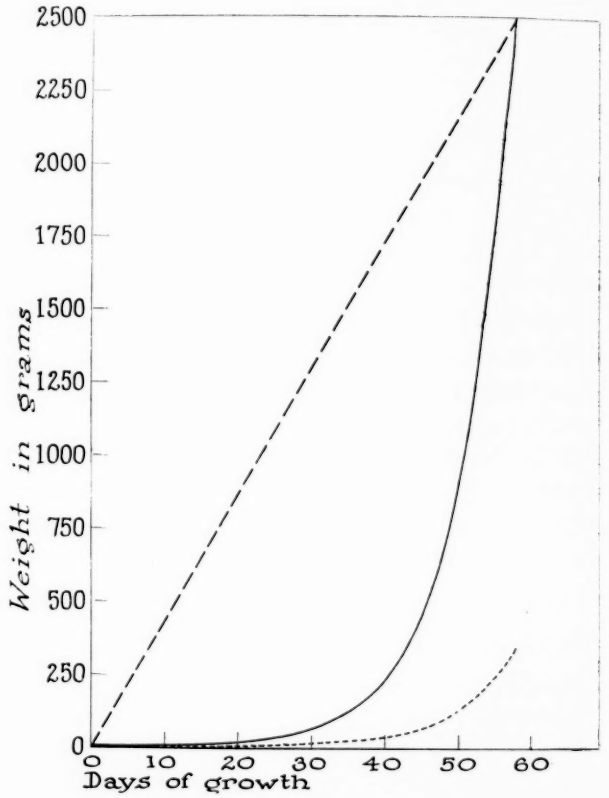


FIG. 1.—Estimation of the rate of increase in mass exhibited by the tumor. Broken line (— — —): weight of tumor mass at constant rate of 43 gm. per day; solid line(——): weight as given by equation I; broken line (– – –): corresponding rate of gain (gm. per day).

The smooth curve is that of equation I and shows how the tumor mass may be expected to have increased in weight under conditions of "free" reduplication. The chief gain must thus have occurred after, rather than before, the 45th postoperative day.

The rate of 100 gm. per day increase would not have been reached until about the 50th day when the mass itself weighed about 850 gm.

For most of the period of growth the left side of the thorax was drained. Hence, mechanical and physical conditions were good for free; *i.e.,* unhampered growth. Although necrosis was not a conspicuous feature of the tumor mass, it was present in small amounts. Thus some cells could not multiply and the curves must be understood to represent simply an approximation to the true course of events.

Discussion

In addition to Bohrod's paper, referred to above, the studies of Schrek (2) require comment. Of especial significance is his observation that the Walker rat tumor increases in size at a rate comparable to, but definitely not greater than that which is exhibited by the rat embryo. Apart from certain differences of method in handling data on growth, the question which Schrek's findings raise may be touched upon here, as a matter of interest, though not as one of demonstration, owing to the lack of serial observations in our case. Considering the results as they stand, however, we find the rate of growth of the pulmonary cancer to have been 0.135 gm. per gram per day and hence the time to double a given mass of tumor as 5.14 days, approximately. These results, as indicated above, are not only quite plausible under the assumption of free and unhampered reduplication, but they are also consistent with the facts that a negligible portion remained *in situ* at operation; that the pleural cavity appeared to be "partially" filled with tumor tissue on the occasion of the rib resection (50th day), and that the mass at autopsy actually weighed 2,500 gm. Now, a comparison of the tumor growth rate as given by α in equation I; namely, 0.135 gm. per gram per day, with that of the human embryo between the 45th and 50th menstrual days as estimated from Streeter's (3) data on weight, shows that both are practically identical. While the data for this very early embryonal period are scant, there are at least sufficient observations to indicate that embryonal growth is even slightly greater between the 40th and 45th days, though it is, on the other hand, measurably smaller in the immediately succeeding period between the 50th and 60th days, during which the embryo requires about 6 days to double its mass rather than the 5 days estimated for the tumor. The foregoing results are thus in harmony with Schrek's observation on the identity of growth rate of rat tumor and embryo and they offer an additional reason for pursuing the question of whether or not the growth rate of malignant tumors actually approaches the greatest homologous embryonal values as a limit. The curve of rate of free reduplication calculated for this pulmonary tumor gives a rate that practically equals that of very early embryonal life. It may be remarked, finally, that the rate for the fetus is much less than that for the embryo and that the rate for the tumor in this case is about 16 times that of the human infant at birth.

Summary

Following pneumonectomy in a white male 39 years old, with no residual tumor observed in the thorax at the time of operation, a bronchiogenic carcinoma simplex attained a weight of 2,500 gm. at the time of death 58 days later. Metastases were found in regional lymph nodes, pericardium, and veins of esophagus, together with direct extension to the subcutaneous tissues beneath the surgical incision. Computation shows that the growth of the tumor must have been much more rapid in the later days than in the earlier days, probably attaining an ultimate value of about 340 gm. per day.

REFERENCES

1. Bohrod, M. G. Pleural Mesothelioma with Unusual Rate of Growth. Am. J. Clin. Path., **11**:60-67. 1941.
2. Schrek, R. A Comparison of the Growth Curves of Malignant and Normal (Embryonic and Postembryonic) Tissues of the Rat. Am. J. Path., **12**:525-530. 1936.
3. Streeter, G. L. Weight, Sitting Height, Head Size, Foot Length, and Menstrual Age of the Human Embryo. Carnegie Inst., Washington, Pub. No. 274, Contrib. Embryol., **11**:(No. 55) 143-170. 1920.
4. Wetzel, N. C. On Motion of Growth; Theoretical Foundations. Growth, **1**:6-59. 1937.

Influence of Heptaldehyde on Pregnancy in Rats

C. Carruthers, Ph.D., and R. E. Stowell, M.D.

(From the Research Department of The Barnard Free Skin and Cancer Hospital, St. Louis, Missouri)

(Received for publication June 16, 1941)

The observations of Strong (14, 15) and of Boyland and Mawson (1) that heptaldehyde caused regression of spontaneous mammary tumors in mice have aroused the interest of oncologists. Carruthers (3) found that heptaldehyde inhibited the production of epitheliomas by methylcholanthrene and caused atrophic and fibrotic changes in the skin of mice. Strong described liquefaction and hemorrhages in spontaneous mammary tumors.

Supplementing work on the anticarcinogenic action of heptaldehyde, it seems desirable to study its action upon embryonic tissue, which possesses some properties similar to rapidly growing tumors. Therefore these experiments were undertaken to study the influence of heptaldehyde on pregnancy in the rat. Heptaldehyde does not act specifically on neoplastic tissue, for Strong (13) and Carruthers (4) found it caused resorption of embryos in pregnant mice. Carruthers obtained resorption of mouse embryos by administering heptaldehyde orally and more efficiently by injecting it intraperitoneally.

Experimental Methods

For these experiments Wistar, piebald, and stock rats were used. Several young females of 150 to 200 gm. were mated with one male and the success of mating and the progress of pregnancy was followed by making daily vaginal smears. Because of its usual early appearance in resorptions, attention was devoted to the blood or placental sign, the bloody vaginal discharge associated with seepage of blood from the placenta (normally observed on the 13th day of pregnancy in the rat).

In addition to daily weights of rats, records were made of the *initial* change in weight; *i.e.*, during the first 5 days of pregnancy, and of the *final* weight change; *i.e.*, the difference between the weight on the day of insemination and immediately after parturition. The rats were used only once to study the influence of heptaldehyde on pregnancy, unless the effect of a resorption upon subsequent pregnancy and lactation was to be observed. Purina Dog Chow and water were allowed ad libitum.

In a preliminary experiment, 0.5 cc. of heptaldehyde administered daily by stomach tube throughout pregnancy in total dosages up to 10 cc. did not cause resorption of embryos in 10 rats. Since it has been demonstrated by Carruthers (4) that the resorption of embryos in the mouse can be induced by the oral administration of heptaldehyde, the failure to produce resorptions in the rat was attributed either to destruction of the heptaldehyde or to its poor absorption from the gastrointestinal tract of the rat. Since oral administration was unsatisfactory, intraperitoneal injections were made of heptaldehyde dissolved in an equal volume of acetone or in the fraction of Armour's "Pure Leaf" lard melting at or below 37° C. (2). The heptaldehyde used in all these experiments was obtained from the Eastman Kodak Co., redistilled every 2 to 3 weeks, and stored at 0-5° C. Daily intraperitoneal injections were started on either the day of, or one day after, insemination (determined microscopically by finding sperm in the vaginal smear) and continued until the pregnancy was terminated by resorption of embryos or parturition.

Since Strong (14, 15) has shown that a combination of 3 parts of heptaldehyde and 1 part of methyl salicylate was more effective in bringing about liquefaction, and in some cases, complete regression of spontaneous tumors in mice than was either chemical alone, synthetic methyl salicylate was added to the heptaldehyde in a concentration of 0.1 per cent or of 1.0 per cent to stabilize it. Strong believed the enhanced activity of heptaldehyde and methyl salicylate was due to the presence of traces of antioxidants in the latter.

Results

Production of resorptions.—Some of the effects of heptaldehyde in resorptions in individual rats and its effect upon their subsequent reproductive ability are shown in Table I, (a) and (b). Heptaldehyde is toxic when injected intraperitoneally, but such manifestations are usually less evident if the rats are conditioned by initial small injections. Usually, for this part of the experiment, 0.2 cc. of lard or of acetone solution containing 0.1 cc. of heptaldehyde was injected for the first few days, after which the dosage was doubled and rarely trebled. The heptaldehyde was injected daily despite weight changes, the effective total dosage ranging from 1.3 to 3.7 cc. Five rats had uninterrupted gestations although they received 1.7 to 3.5 cc. of

heptaldehyde. Seven of the 12 rats had resorptions, although 2 of these lost 16 gm. which may have affected the course of pregnancy. Rat No. 49 had a successful gestation after losing 20 gm. After resorptions 3 of the 7 rats had subsequent successful gestations. This observation has been confirmed in other experiments, indicating that heptaldehyde does not materially affect the reproductive system of the rat. In rat No. 21 the estrus cycle was disrupted for some time, but eventually a pregnancy followed. Rat No. 23 died 11 days after resorption and autopsy showed ascites.

placental sign varied from 7 to 12 days, the average being 10 days.

Nine rats receiving 1.0 to 1.6 cc. of heptaldehyde had successful gestations (Table II, (b)), and reared normal young. The placental sign occurred an average of a day later than in rats having resorption of embryos.

The control group consisting of 11 rats (Table II, (c)), injected with lard alone and lard containing 1 per cent methyl salicylate, all had uninterrupted gestations. The placental sign occurred at an average of 11½ days.

TABLE I: THE EFFECT ON PREGNANCY IN THE RAT OF THE INTRAPERITONEAL INJECTION OF HEPTALDEHYDE IN LARD

Rat No.	Heptaldehyde injected in cc.	Concentration of methyl salicylate, per cent	Occurrence of placental sign in days	Loss or gain in body weight		Subsequent history
				Initial	Final	
(a) Unsuccessful gestations:						
21	3.1	1	8	0	0	E.C.D.; S.G.
35	2.4	1	7	0	0	E.C.R.; S.G.
32	2.2	1	9	−16	−20	E.C.R.; S.G.
23	3.7	1	8	−16	+ 2	E.C.D.; ascites
20	2.2	1	10	0	+20	E.C.R.; S.G.
57	1.3	0	10	0	+14	E.C.R.
69	2.7	0	11	0	0	E.C.R.
(b) Successful gestations:						
31	3.5	1	12	0	..	E.C.R.
25	2.4	1	9	0	..	W.
27	2.8	1	10	0	..	W.
62	1.7	0	11	0	..	W.
49	2.2	0	11	−20	..	W.
(c) Unsuccessful gestations:						
1-4	1.9-2.3 (2.1)	0	10-13 (10.7)	0-8 (−4)	..	S.
5-7	2.7-3.1 (2.9)	0	11-13 (11.6)	0-10 (−3.3)	..	S.
(d) Successful gestations:						
8-9	2.5-2.7 (2.6)	0	14-15	0-22	..	S.

Average data are indicated in parenthesis. Subsequent histories are denoted by E.C.D., estrus cycle disrupted; S.G., successful gestation; E.C.R., estrus cycle returned; W., withdrawn from experiment; S., sacrificed for gross and histological examination.

The results of using lard and acetone as solvents for heptaldehyde and of decreasing the effective resorptive dose of heptaldehyde are shown in Table II, (a) and (b). In this part of the experiment usually 0.025 to 0.05 cc. of heptaldehyde in 0.05 to 0.1 cc. of lard or of acetone were injected in each rat daily for the first 3 or 4 days, then the dose was increased to 0.1 cc. for the next 2 to 4 days. Later the dose was sometimes increased to 0.2 cc. per rat per day, but it never exceeded 0.3 cc., a dose which was seldom used.

Acetone (A.R.) was used as a solvent for heptaldehyde in an attempt to avoid the ascites which developed in some of the lard-injected rats. The effective resorptive dose varied from 0.20 to 1.8 cc. of heptaldehyde in lard and from 0.25 to 1.6 cc. in acetone. The smallest amount of heptaldehyde capable of interrupting pregnancy was 0.2 cc. The appearance of the

Examination of rats.—In an attempt to determine the mechanism of resorption of rat embryos following administration of heptaldehyde, macroscopic and microscopic examinations were made on 29 pregnant rats of which 13 showed resorptions and 8 were normal controls receiving no injections (Tables I, (c) and (d), and II). Since the date of resorption in the 13 rats as evidenced by the first appearance of bloody vaginal discharge varied between the 10th and 15th day, it was impossible to predict accurately the exact date at which any specific rat might initiate resorption of embryos. Therefore, in addition to killing animals with chloroform at 2 day intervals from 6 to 18 days after insemination, additional animals were killed at the first appearance of the blood sign.

The rats treated with heptaldehyde in lard injections showed varying degrees of ascites, of whitish exudates

coating liver and spleen, and of adhesions, chiefly of liver to diaphragm and to intestine and of ovaries to intestine. Most of the rats injected with heptaldehyde in lard showed hemorrhages in the lungs after resorption of embryos. In a few cases the lungs were chocolate-colored. In rats receiving injections of heptaldehyde in acetone, lung hemorrhages were not seen and peritoneal changes and ascites were much less pronounced. The uteri of rats killed at the time of resorption were filled with blood between implantation sites, and the uterine vessels were dilated.

Microscopic examinations were made of the uterus, ovaries, kidney, spleen, liver, and lung fixed in Bouin's fluid, and of adrenal, thyroid, and pituitary fixed in Zenker formol solution. The uterus was fixed *in toto,* cut into suitable lengths, and representative longitudinal or cross sections made through each conceptus and implantation site.

The mechanism of implantation appeared normal. In the early stages of resorption, there is dilation of the sinuses of the trophoblast followed by hemorrhage into the fetal membranes, decidua capsularis, and placenta with some escape of blood into the uterine lumen (Fig. 1). This hemorrhage is associated with the early blood sign. Death of the embryo is followed by disintegration of the embryo and placenta. Subsequently there is disintegration of the decidua subplacentalis often accompanied by the invasion of phagocytic cells, chiefly polymorphonuclear neutrophils. In a few days the fetal and placental tissues slough into the uterine lumen (Fig. 2). Finally, there is only a remnant of decidua subplacentalis infiltrated with polymorphs which disappears leaving macrophages filled with hemosiderin in the myometrium beneath the site of resorption. Sometimes, as is shown in Fig. 2, the degree of resorptive changes varied greatly between adjacent embryos. During resorption the hemosiderin content of the fetal and maternal blood is greatly increased. The lungs of lard-injected rats showed that the hemorrhages were associated with a subacute interstitial lipoid pneumonitis. In nonpregnant control rats injected intraperitoneally with lard which did not contain heptaldehyde or methyl salicylate, many lipoid-filled macrophages were demonstrated in frozen sections of the lung stained with Sudan IV. It seems probable that the observed hemorrhages and pneumonitis were caused by the irritating action of foreign animal fat, as has been described by others, and were not associated with a specific action of the heptaldehyde. Microscopic examination of other organs did not reveal any abnormalities except for the coating of fibrin and thickening of the capsule chiefly on the liver and spleen.

Smears of the peritoneal exudate were negative for bacteria. Blood studies including stained smears, platelet counts, and determinations of bleeding time and clotting time showed no abnormalities.

TABLE II: THE EFFECT ON PREGNANCY IN THE RAT OF THE INTRAPERITONEAL INJECTION OF HEPTALDEHYDE IN LARD AND IN ACETONE WITH METHYL SALICYLATE

No. of rats	Heptaldehyde injected in cc.	Solvent for heptaldehyde	Occurrence of placental sign in days	Change in body weight in gm.	
				Initial	Final
(a) Unsuccessful gestations:					
9	0.20-0.73 (0.55)	Lard *	9-11 (9.6)	0-8 (—3.5)	(+5)
3	1.00-1.12 (1.04)	Lard *	10-11 (10.3)	0-8 (—2.6)	(+10)
4	1.30-1.60 (1.40)	Lard *	8-10 (9.0)	0-8 (—2.0)	(+9)
1	1.80	Lard *	9	—6	+16
4	0.25-0.40 (0.34)	Acetone †	8-11 (10.0)	0-6 (—3.5)	....
1	0.70	Acetone *	11	0	....
6	0.70-1.10	Acetone †	7-12 (9.0)	0-4 (—1.0)	(+9)
1	1.60	Acetone †	11	—1.4	—2
(b) Successful gestations:					
3	1.00-1.10 (1.10)	Acetone *	9-12 (10)	0-10 (—5)	....
2	1.60 (1.60)	Acetone *	11-15 (13)	0-6 (—3)	....
4	1.40-2.00 (1.60)	Acetone †	9-11 (10)	0-6 (—2)	....
(c) Successful gestations—lard controls:					
1	0	0.6 cc. lard	12	2	....
4	0	1.3-2.6 cc. lard	11-13 (11)	0	....
6	0	1.4-3.3 cc. lard *	9-13 (12)	0-2	...

Average data are indicated in parenthesis. Concentration of 1.0% methyl salicylate is indicated by *; 0.1% by †.

DISCUSSION

The action of heptaldehyde is not specific or uniform for any one type of tissue. The dosage of heptaldehyde necessary to cause resorption of embryos in individual rats varied greatly, was equally effective in lard or in

acetone, and slightly more effective with 0.1 per cent than with 1.0 per cent methyl salicylate. Usually weight changes were of little significance. Many rats had a slight decrease of weight during the first few days of treatment, but the weight after parturition was usually slightly greater than the initial weight, indicating that the heptaldehyde was tolerated satisfactorily. In rats undergoing resorption of embryos, the placental sign occurred in some cases as early as 7 days and averaged 9 to 10 days. Occasionally the placental sign was of one day's duration and not striking, while more often the blood flow was copious in some sections, hypertension was considered as a cause of the hemorrhages. Rytand and Dock (11, 12) have described a correlation between increase in blood pressure and increase in heart weight in rats. No significant alteration in the ratio between the body weight and either the weight of the entire heart or the weight of the ventricles was found in these rats. However, within the 18-day experimental period there might not be sufficient time to permit compensatory hypertrophy of the ventricular muscle.

No abnormality or deficiency was found in the blood or in its clotting mechanism to account for uterine

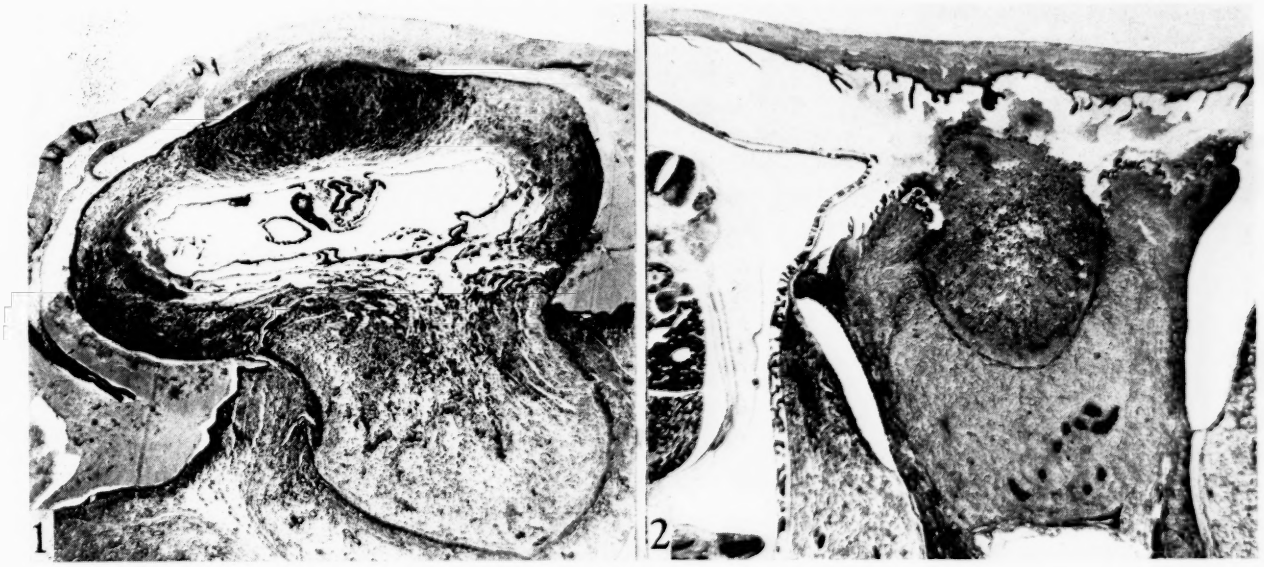


FIG. 1.—Photomicrograph of section of pregnant uterus of rat showing early stage of resorption of embryo following injections of heptaldehyde during 10 days after insemination. Hemorrhage has occurred into the decidua capsularis and uterine lumen. The cells of the embryo appear dead. Bouin fixation; hematoxylin and eosin stain. Mag. × 15.

FIG. 2.—Photomicrograph of section of pregnant uterus of rat, showing late stage of resorption of embryo following injections of heptaldehyde during 16 days after insemination. Fetal and placental tissues are sloughing into the uterine lumen. Two adjacent embryos appear to be viable. Bouin fixation; hematoxylin and eosin stain. Mag. × 8.

and lasted for several days before the estrus cycle reappeared.

Intraperitoneal injections of heptaldehyde dissolved in lard produced changes such as ascites, deposition of fibrin, and adhesions of organs. These evidences of peritoneal irritation and the negative smears for bacteria suggest the production of a chemical rather than a bacterial peritonitis. Although peritoneal irritation through the initiation of uterine muscular contractions conceivably might cause death of embryos, intraperitoneal injections of various irritating agents were not observed to interfere with gestation. The resorption of embryos in progesterone deficiency may give changes somewhat similar to those described here. There is a contraction of uterine muscle which interferes with the blood supply to the fetal placenta followed by fetal death and resorption.

Because of the capillary and venous dilation observed hemorrhages. No degree of disturbance of fetal hematopoietic activity such as Evans and Burr (5) described in vitamin E deficiency was observed, nor was there definite evidence of vitamin A deficiency, which Mason (10) has described as causing resorption of embryos. Kudryashov and his co-workers (6-9) found that rancid pig fat, aerated oleic acid, and wheat germ oil treated with ethereal ferric chloride administered orally or by subcutaneous injection caused resorption of embryos in female rats. They believed that the active constituent was the aldehyde or ketone fraction and that it did not act by destroying vitamin E in the tissues.

SUMMARY

1. Heptaldehyde dissolved in lard, in lard containing 1.0 per cent or 0.1 per cent methyl salicylate, and in acetone containing 0.1 per cent methyl salicylate is

capable of inducing the resorption of rat embryos when injected intraperitoneally for 7 to 20 days beginning on the day of or one day after insemination. Heptaldehyde administered by stomach tube is ineffective.

2. Heptaldehyde injected intraperitoneally is toxic, but the effect becomes slight, or not apparent if the animal is conditioned by preliminary small doses which are gradually increased.

3. The reproductive system of the rat apparently is not impaired by heptaldehyde since many rats after resorptions had subsequent normal gestation.

4. In rats undergoing resorption of embryos, the placental sign occurred an average of 3 days earlier, was usually more profuse, and was the best indication of the onset of resorptive changes.

5. Histologically the resorptions do not appear to be characteristic of vitamin A or vitamin E deficiency. It seems probable that heptaldehyde must bring about resorption of rat embryos through some physiological mechanism for which little anatomical evidence has been observed, other than hemorrhage from and death of the placental tissues.

Addendum

Recently Deatherage, *et al.* have reported that they were unable to affect reproduction in female rats by the oral, subcutaneous, or intraperitoneal administration of rancid animal fat, their volatile oxidation products, the unsaponifiable portion of irradiated fats and of some aldehydes. (See: Deatherage, F. E., K. P. McConnell, and H. A. Mattill. Proc. Soc. Exper. Biol. & Med., **46**:399-402. 1941.)

References

1. Boyland, E., and E. H. Mawson. Experiments on the Chemotherapy of Cancer. II. The Effect of Aldehydes and Glucosides. Biochem. J., **32**:1982-1987. 1938.
2. Burrows, H., I. Hieger, and E. L. Kennaway. The Experimental Production of Tumors of Connective Tissue. Am. J. Cancer, **16**:57-67. 1932.
3. Carruthers, C. Aldehydic Resorption in Mice. Proc. Soc. Exper. Biol. & Med., **41**:336-340. 1939.
4. Carruthers, C. Influence of Heptaldehyde on Carcinogenic Action of Methylcholanthrene. Arch. Path., **30**:1184-1191. 1940.
5. Evans, H. M., and G. O. Burr. The Antisterility Vitamine Fat Soluble E. Memoirs Univ. of Calif., **8**:1927.
6. Kudryashov, B. A. Abwendung und Liquidation der Schwangerschaft mittels der Fettabbauproducte. Arch. f. exper. Path. u. Pharmakol., **169**:275-289. 1932.
7. Kudryashov, B. A., and H. Benjajeva. Die Wirkung von Abbauproducten der Fette auf den graviden Uterus der Ratte. Arch. f. exper. Path. u. Pharmakol., **175**:489-499. 1934.
8. Kudryashov, B. A., and P. A. Agatov. Sterilization by Means of Decomposed Fat. Ginekologia i Akusherstvo, **6**:1. 1935.
9. Kudryashov, B. A. A Critical Analysis of the Waddell and Steenback Effect Observed in the Ferric Chloride Treatment of Fodders. Bull. biol. et. méd. expér. de l'URSS, **6**:220-221. 1938. Chem. Abstracts, **33**:3428. 1939.
10. Mason, K. E. Foetal Death, Prolonged Gestation, and Difficult Parturition in the Rat as a Result of Vitamin A Deficiency. Am. J. Anat., **57**:303-349. 1935.
11. Rytand, D. A., and W. Dock. Experimental Concentric and Eccentric Cardiac Hypertrophy in Rats. Arch. Int. Med., **56**:511-520. 1935.
12. Rytand, D. A. The Renal Factor in Arterial Hypertension with Coarctation of the Aorta. J. Clin. Investigation, **17**: 391-399. 1938.
13. Strong, L. C. The Treatment of Spontaneous Tumors in Dogs by the Injection of Heptyl Aldehyde. Science, **88**: 111-112. 1938.
14. Strong, L. C. The Synergistic Effect of Heptyl Aldehyde and Methyl Salicylate on Spontaneous Tumors of the Mammary Gland in Mice. Yale J. Biol. & Med., **11**:207-218. 1939.
15. Strong, L. C. Problems of Tissue Specificity and Chemotherapy of Cancer. Am. J. Cancer, **38**:243-252. 1940.

American Association for Cancer Research, Inc.

34th Annual Meeting

Hotel Stevens, Chicago, Illinois, April 14-16, 1941

Proceedings of Scientific Sessions

TUESDAY MORNING SESSION*—APRIL 15

DR. BURTON T. SIMPSON, PRESIDENT, PRESIDING

STUDIES ON THE ROUS VIRUS IN FOREIGN SPECIES. F. DURAN-REYNALS. (Yale University School of Medicine, New Haven, Conn.)

Newborn ducklings were infected with the Rous virus of chicken sarcoma by intravenous injection of large amounts of virus. The infection produced either a fatal hemorrhagic disease, unaccompanied by tumor formation, similar to that seen in newborn chicks injected with the virus, or the development of late tumors. The late tumors consisted of one or a few slow-growing sarcomas in the skin, digestive tract, skull, ribs, and muscles. Occurring months after the injection when the bird is full-grown these tumors may well give the impression of spontaneous neoplasms. The virus which acquired the capacity to infect ducks lost its capacity to infect adult chickens. Only young chicks were susceptible to the duck virus. Immediate and late tumors were produced by the duck variant of the virus after injection into chicks. The late tumors in chickens occurring 2 to 10 months after injection of the virus consisted of one or a few sarcomas, usually in bones, often combined with secondary proliferation of bone. These lesions were identical with the so-called osteoporosis often seen in association with leukosis in chickens. The variant of the virus was produced suddenly in the first passage in the duckling.

A preliminary report was published in *Science*, **93**:501-502. 1941. The complete paper will be published in *Cancer Research*.

STUDIES ON THE TRANSMISSIBLE AGENT OF ROUS CHICKEN SARCOMA I. ISOLATION OF VIRUS FROM BASIC PROTEIN-VIRUS COMPLEX. DAVID SHEMIN (by invitation), E. E. SPROUL, and J. W. JOBLING. (College of Physicians and Surgeons, Columbia University, New York, N. Y.)

The basic protein, papain, was added to an extract of the Rous sarcoma which had been treated with hyaluronidase to lower the viscosity. The precipitate which formed immediately contained all of the active material. From a filtrate which was active to a concentration of 7×10^{-5} mgm. N, the precipitate was active to a concentration of 2×10^{-5} mgm. N, while the supernatant fluid was inactive. This precipitate or papain-agent-complex was purified further by solution in 3 per cent NaCl and reprecipitation by addition of distilled water. After several repetitions of this process the final precipitate obtained was white and highly active. This material was not homogeneous. It contained papain and other components. In the Tisselius electrophoresis apparatus, with a solution at pH 7.5, the papain migrated toward the cathode and several other components toward the anode. A pellet obtained by differential high-speed ultracentrifugation of the solution in 3 per cent NaCl was more homogeneous. This material had a migration velocity of $4.9\times10^{-5}\ \frac{\text{cm./5 cc.}}{\text{volts/cm.}}$ in the Tisselius apparatus.

The yield calculated in terms of N was about 0.5 per cent. From an extract containing 1.0 gm. of N which was active to a concentration of 2.8×10^{-5} mgm. N, the final fraction containing 5.0 mgm. N was isolated. This was active to a concentration of 7×10^{-7} mgm. N. Analysis of final fraction yielded the following results: N, 7.2 per cent; P, 1.6 per cent; ash, 2.6 per cent; lipid, 41.0 per cent; N (nonlipid), 11.2 per cent; purine, 2.1 per cent of total N. If it is assumed that the purine found is part of a tetranucleotide, the following may be calculated: nucleic acid, 1.4 per cent; nucleic acid P, 11.0 per cent of total P.

Although it cannot be stated definitely that this active material, which is quite similar to that of Claude and Pollard, is the virus, isolation of it by another method gives some support to the idea that the sarcoma virus is of a more complex nature than the plant viruses.

Discussion

J. F. FURTH (New York, N. Y.): Has Dr. Schemin tried to isolate by a similar procedure a similar substance from the normal tissues?

D. SHEMIN: This attempt has not been made with normal tissues or other animal tumors.

J. FURTH: Until that is done no claim can be made that this represents a pure virus preparation because it is possible that similar material, but without virus activity, may be derived from normal tissues.

D. SHEMIN: No claim is made that the material is pure. Similarity of materials does not mean that they are identical in activity. There may be differences of activity in similar materials. The question of precursor substances also is to be considered.

C. VOEGTLIN (Washington, D. C.): Have the results with the Tisselius apparatus been supplemented with observations made by use of the optical centrifuge?

*Dr. George M. Smith, Vice-President, presided at the meeting of a separate section of this session.

D. SHEMIN: On account of technical difficulties, this was not possible at the time when the only available sample of 70 mgm. of purified material was being studied.

THE ENDURING PARTNERSHIP OF A NEOPLASTIC VIRUS WITH TUMOR CELLS: EXPERIMENTS WITH THE V2 RABBIT CARCINOMA. JOHN G. KIDD. (Rockefeller Institute for Medical Research, New York, N. Y.)

Experiments already reported have shown that the papilloma virus or some variant of it persists enduringly in the transplanted V2 carcinoma of rabbits—a squamous cell carcinoma derived originally from a virus-induced papilloma (*J. Exper. Med.*, **71**:813. 1940). The virus continues to increase in the V2 carcinoma even when this is transplanted in series in animals previously rendered immune to the virus, and the tumor grows as well in virus-immune animals as in comparable normal controls. Manifestly the association of virus and cancer cells is such that neither is in the least affected by antiviral antibody *in vivo*. The findings contrast significantly with those obtained by workers studying the extraneous viruses which ride along as passengers in transplanted tumors. For the passenger viruses do not cause cellular proliferation, and they can be eliminated by passing the tumors through a succession of hosts previously rendered immune to them.

To be published in the *Journal of Experimental Medicine*.

EARLY CANCER OF THE STOMACH. A STUDY OF 1,299 RESECTED ULCERS AND 2,408 CANCERS. WM. C. MacCARTY, SR. (Mayo Clinic, Rochester, Minn.)

Published in *Cancer Research*, **1**:536-537. 1941; abstract p. 590.

Discussion

S. WARREN (Boston, Mass.): Has Dr. MacCarty ever seen an ulceration on the greater curvature of the stomach which has not been a malignant process? What proportion of these 1,299 resected ulcers represent ulcers treated in the Mayo Clinic?

WM. C. MacCARTY: I have seen ulcers on the greater curvature, chronic ulcers in fact, that were not cancer.

In answer to the second question: All of the ulcers we see, whether they are malignant or not, have been treated by someone, and some were treated medically in the Mayo Clinic. We do not operate upon all ulcers of the stomach. In this whole series there were 1,299 resected ulcers which we thought might be cancer, and during the same period there were 2,408 cancers.

Dr. MacCarty cited the cases of a pioneer roentgenologist and a distinguished surgeon in whom the ulcerative lesions of the stomach were not discovered to be cancer until the condition was inoperable. He concluded by emphasizing the importance of yearly x-ray examination and early diagnosis.

THE TREATMENT OF LEUKEMIA WITH RADIOACTIVE PHOSPHORUS. SHIELDS WARREN. (New England Deaconess Hospital, Boston, Mass.)

The value of temporarily radioactive phosphorus in the therapy of leukemia has been clearly demonstrated by Lawrence and his co-workers. The series of cases here reported is presented to demonstrate the results of its use chiefly in subacute and acute cases of the disease.

Fifteen cases are reported, of which 2 represent chronic myelogenous leukemia, 2 represent terminal chronic leukemia, 4 represent subacute leukemia, and 7 acute leukemia. Four of these cases were moribund when treated and died within a week subsequently. Of the 8 subacute and acute cases living over one week following the first treatment, 5 are living and discharged from the hospital, 2 are dead of leukemia, and 1 dead of pneumonia 9 months after his first treatment.

The usual dosage for adults has been 2 millicurie equivalents of radioactive phosphorus, administered intravenously with 5 per cent glucose and 0.85 per cent saline solution in most cases.

On the basis of these cases of leukemia treated with artificially radioactive phosphorus, it may be said: 1. No harm has been done the patients with careful control of the amounts used. 2. No instance of radiation sickness has been noted. 3. The white blood cell count may be reduced as efficaciously as with x-ray radiation. 4. The differential count can be brought in most cases toward normal. 5. Erythropoiesis is not interfered with to a greater degree than in the course of the untreated leukemia. Later the red cell count becomes more normal.

Discussion

L. F. BLEYER (Elmira, N. Y.): How long does the improvement in the blood count last with this treatment?

S. WARREN: It is not possible to say how long it will last. In none of the patients who are now living and comparatively well has there been an appreciable failure in the red count once it has become re-established. It usually takes about three or four months for a stabilization of the red count. These cases have not gone very long as yet; the longest period is a little over a year.

CHEMICAL CONFIGURATION AND CARCINOGENESIS. CHARLES E. DUNLAP and SHIELDS WARREN. (Collis P. Huntington Memorial Hospital and Harvard Medical School, Boston, Mass.)

We have been testing the activity of 65 new derivatives of the four best known carcinogenic hydrocarbons containing in common the 1,2-benzanthracene nucleus. The chief test has been of the ability of the compounds to produce tumors at the site of injection in mice. These derivatives were prepared by Dr. Louis Fieser and his associates. We have been able to confirm the previous observation that substitution products of 1,2-benzanthracene in the 9, 10, 5, and 6 positions are the most likely to be carcinogenic. Very slight changes in the structure or configuration of the compound are followed by great changes in carcinogenic activity.

In the benzpyrene group the 9-methyl derivative produces tumors in 85 days, the 6-methyl in 160 days, while the 2′,3′-methyl derivatives have, so far, produced no tumors. While the 6-methyl derivative is quite active, the 6-hydroxy derivative has little if any activity. The great majority of changes in structure or configuration inacti-

vate the compounds completely. The ability to produce tumors is confined to a relatively small number of specific chemical substances within this family and is not a general characteristic of the whole group.

Carcinogenic activity is directly dependent on the chemical structure of these compounds and presumably must involve a fairly specific set of physicochemical reactions between the carcinogenic agent and the tissues. In studies of the possible interactions tests have been made of a number of compounds prepared principally by Dr. Erich. These are chemical combinations between a carcinogenic nucleus and amines, proteins, and other organic radicals containing oxygen and nitrogen. A few have proved active, but they are generally weaker than the parent compound. Only one, 1,2,5,6-dibenzanthryl-9-isocyanate has produced tumors somewhat more rapidly than its parent compound. It is improbable that this compound could be formed from dibenzanthracene in the body.

Hydroxy derivatives of the types which may be excreted by an animal after injection of these carcinogenic compounds have been tested. These have included 6-hydroxy-3,4-benzpyrene, and 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene. In test periods extending over 1½ years and 7 months, respectively, these compounds have shown little, if any, activity.

A new compound, 4,9-dimethyl-5,6-benzthiophanthrene may prove to be particularly useful for following directly the chemical changes undergone by a carcinogenic agent at the site of injection in the animal. This compound, synthesized by Sandin and Fieser, produced tumors in 116 days. It has about the same potency as its nearest relative in the benzanthracene series, 9,10-dimethyl-1,2-benzanthracene. From the chemical point of view: 1. A new carcinogenic nucleus has been demonstrated which contains only 3 benzene rings. 2. Sulfur has been incorporated in the ring system. 3. The traditional benzanthracene nucleus has been shown to be nonessential for high potency compounds. From the experimental point of view: the presence of sulfur in the ring system would permit the substitution of radioactive sulfur, to serve as a tracer in following the metabolism of this substance during tumor induction.

Discussion

E. T. BELL (Minneapolis, Minn.): Do the inactive compounds produce as severe local inflammatory reactions as the carcinogenic compounds?

C. E. DUNLAP: Almost all of these compounds produce some degree of irritation at the site of injection. The irritation produced is not correlated with the carcinogenic activity. Some of the inactive compounds produce such irritation that ulceration results. Many of the extremely active compounds produce only slight irritation.

EFFECT OF A CONCENTRATE FROM B. PRODIGIOSUS FILTRATE ON SUBCUTANEOUS PRIMARY INDUCED MOUSE TUMORS.

M. J. SHEAR. (National Cancer Institute, Bethesda, Md.)

The earlier work of Gratia and Linz and of Shwartzman and Michailovsky, with crude bacterial filtrates, led us to attempt to separate the hemorrhage-producing agent from inactive and toxic contaminants. In collaboration with Dr. Andervont, broth cultures of the colon bacillus were employed; then, with Dr. Turner, broth cultures of *B. prodigiosus* were used. Fractions were obtained from filtrates of both organisms which produced hemorrhage in transplanted sarcomas in doses as low as 0.4 microgram. These fractions gave a negative biuret test for protein and a strongly positive Molisch test for carbohydrate.

It was reported by Shear and Turner last year that the difficulty of removing the troublesome contaminants contributed by the constituents of the original broth medium itself could be avoided by employing a simple medium; that concentrates were obtained which regularly produced hemorrhage in a dilution of 1:10,000; that the agent was stable in solution for many months; that it was not dialyzable through cellophane between pH 1 and 10; and that the concentrates were about 1,000 times as potent as a commercial preparation of "Coley's mixed toxins."

The results of micro-analyses by the Arlington Laboratories show that, although the figures for the active fraction are similar to those of some other nitrogen-containing polysaccharides, it is not yet possible to say whether the active agent is a polysaccharide, or whether it is merely accompanied by polysaccharide contaminants.

This concentrate was administered to some 300 strain A mice bearing primary subcutaneous tumors induced by benzpyrene. As far as complete regression of the tumors is concerned, the results were largely negative. Nevertheless, the peculiar selectivity of this material for sarcoma tissue justifies further attention.

Histological observations were not carried out in these particular experiments since the tumors, with few exceptions, grew progressively until the mice died. However, on the basis of Dr. Stewart's examination of sections of a great number of such induced tumors in our previous experiments, they were probably spindle cell sarcomas for the most part.

Doses more than 500 times the minimum for hemorrhage-production were lethal for most of the strain A mice. Normal mice tolerated larger doses, provided the first dose was not too high, and provided the dosage was not increased at too rapid a rate. Doses of 5,000 to 8,000 units were tolerated.

The same initial dose, of 100 units, was given to 27 mice with primary tumors; 18 were dead the next day. There were fatalities in other experiments with even one-fourth that dose. However, most of the mice killed with such doses were those bearing large tumors.

It was repeatedly observed that mice with large tumors succumbed to a given dose while those with smaller tumors survived. Thus, in addition to the toxicity of the material itself, as shown in mice without tumors, there was apparently an additional toxic effect when tumor was present. Moreover, this added toxicity was greater, in general, the larger the tumor.

Severe hemorrhage was produced in a few hours in these primary tumors. Although necrosis of part of the tumor often followed the hemorrhage, the tumors usually resumed growing. Repeated injections were next tried.

Mice with primary tumors tolerated doses considerably

above the dose lethal in a single injection, provided the first dose was small, and provided the dosage was not increased at too rapid a rate. The behavior of tumor-bearing mice was analogous to that of normal mice in that tolerance to large doses seemed to have been produced in many cases; however, the maximum tolerated doses, both in single and in repeated injections, were less in the case of those with tumors.

In spite of the fact that this material has so far produced regression of only a few of the primary tumors, it is felt that further work with it is warranted. Its selective affinity for sarcoma tissue is striking. Moreover, hemorrhage is not produced in the normal tissues. We know little about the mechanism of its action and it is possible that, in view of its peculiar selectivity, other measures may be found which might enhance its destructive effect on tumors.

Hemorrhage production in mouse tumors has also been reported, by Boyland and by others, with sublethal doses of colchicine, and by Lewisohn with beef spleen extracts. Andervont found that the combined action of colchicine and bacterial filtrate was no more effective than either agent alone.

Various workers have shown that tumor tissue is rich in reducing substances such as glutathione, ascorbic acid, and cysteine; that, following the administration of bacterial filtrate, the ascorbic acid content of tumors is diminished; and that administration of ascorbic acid or cysteine counteracts the hemorrhage-producing action of bacterial filtrate. Recently, we have been trying to destroy or inhibit the reducing action of substances present in tumors. This work is still in its early stages, but it is of interest that maleic acid is also capable of producing hemorrhage in such primary tumors.

Systematic examination of the effect of related substances is in progress.

THE RELATIVE ARGINASE ACTIVITY OF CERTAIN TUMORS AND NORMAL CONTROL TISSUES. J. P. GREENSTEIN, W. V. JENRETTE (by invitation), G. B. MIDER, and J. WHITE. (National Cancer Institute, Bethesda, Md.)

The results on the pairs of tumor and normal control tissues indicate that: 1. the arginase activity of the transplanted hepatic tumors and of the embryonic liver is very much less than that of normal liver; 2. there is no apparent effect of the transplant on the liver arginase of the same animal except in the case of a particularly rapidly growing hepatic tumor in rats; 3. regenerating and normal adult livers have the same activity; 4. the arginase activity of the spontaneous mammary tumors is very much greater, that of the lymphomas only slightly greater, than that of the respective normal control tissues; 5. the activity of the same tissue from different strains is practically identical; 6. the activity of normal lactating and stilbestrol-induced hyperplastic mammary tissues is very nearly the same; 7. the pH optimum of arginase is the same for the tumors and controls; 8. the relative activity of hepatic tumor and liver is not significantly affected by the presence or absence of added manganese; 9. prolonged dialysis, however, of normal liver leads to a preparation which is strongly activated by manganese whereas prolonged dialysis of hepatic tumor extract results in apparently complete loss in enzyme activity; and 10. racemic homoarginine is hydrolyzed to a small extent by normal liver extract, not at all under the same conditions by extracts of hepatic or mammary tumor.

Most of the miscellaneous normal and tumor tissues studied yield relatively low values for arginase activity as compared with that of normal liver and spontaneous mammary tumors, but are of the same order of magnitude as the hepatic tumors and lymphomas. The lowest values are found in bone marrow, brain, and spleen.

The data presented have been published in *J. Biol. Chem.*, **137**:795-796. 1941, and *J. Nat. Cancer Inst.*, **1**:687-706. 1941.

Discussion

M. E. KRAHL (Indianapolis, Ind.): Can the low arginase activity of some of these tumors be raised by the use of cobalt or any other heavy metals which are activators for arginase?

J. P. GREENSTEIN: We used tissue extracts which presumably contain all the natural activators. When we added an excess of manganese to tissues of low activity, such as the liver tumors, there was extremely little increase, certainly less than 1 per cent.

J. C. AUB (Boston, Mass.): Does the arginase content of the liver vary in different nutritional states of the animal; is the arginase curve different in starving animals from that in well-fed animals?

J. P. GREENSTEIN: The only effect of nutrition which we studied was the effect of the high and low cystine diet on animals receiving a supplement containing butter yellow. We have not investigated animals under any other conditions.

METABOLISM OF CHICKEN TUMORS AND LEUKOSES. DEAN BURK, HERBERT SPRINCE, ELVIN A. KABAT, and JACOB FURTH. (Cornell University Medical College, New York, N. Y., and National Cancer Institute, Bethesda, Md.)

Standard metabolic values were obtained with a wide number and variety of types of chicken tumors and with two leukosis strains. Both chemical and manometric methods of analysis were employed, including the Summerson differential manometer for determining all aerobic values with a single sample of tissue. Correlations between metabolic results and histological observations were attempted. It was desired in the main to ascertain whether the chicken sarcomas and leukoses showed typical or possibly quite unusual aspects of metabolism characteristic of cancers in man, rat, and mouse as determined by numerous investigators heretofore; and to make a comparison between the wealth of material now available and that described by Warburg and by Murphy and Hawkins some 16 years ago on the basis of the technics then developed.

The chicken sarcomas show a metabolism highly characteristic of that of cancers observed heretofore, in particular a high anaerobic lactic acid production, a fairly high aerobic lactic acid production, a respiratory quotient

definitely below unity, and approximate equivalence of manometric and chemically measured lactic acid formation. The chicken tumors as a class represent an upper limit of metabolism characteristic of malignant tumor metabolism generally, and they strengthen our previously developed conception, first laid down by Warburg and Dickens, regarding the moderately specific nature of cancer metabolism.

The chicken tumor 1 of Rous, cultured on egg embryo, possesses much of the same metabolism as that grown on chickens, except that the aerobic glycolysis is somewhat lower, and the respiration definitely higher. A transparent, membranous type of embryo-cultured tumor is sometimes formed that exhibits only one-half the aerobic and anaerobic glycolysis of the normal nodular type, and a respiration quotient well above unity, a noteworthy and unique characteristic. This membranous type contained but few tumor cells and was heavily infiltrated with polymorphonuclear leukocytes.

Although the chicken leukoses studied were malignant from the pathological point of view, the metabolic values for leukotic liver, spleen, and bone marrow were not essentially different from their respective nonleukotic control tissues, except in the case of liver. Spleen and hyperplastic bone marrow (produced by acetylphenylhydrazine injections, erythroblastic elements predominating) show a moderate anaerobic glycolysis of themselves, of the same order as the leukosis cells, and hence it is only in the infiltrated liver that the metabolism of the leukosis cells is clearly recognized. The leukosis metabolism, in marked contrast to the chicken tumor metabolism, is probably as little characteristic of malignant tumor metabolism as any malignant material observed so far.

The chemical lactic acid determinations checked practically within experimental error the manometrically determined values of glycolysis, further confirming and establishing the view that the major quantitatively important acid product of malignant neoplasms metabolizing glucose is lactic acid.

Discussion

O. ROSENTHAL (Philadelphia, Penn.): Was there any correlation between the malignancy of the chicken sarcoma and the rate of growth, or was there correlation only between the metabolism and the number of cells?

D. BURK: The metabolism of all the tumors when corrected for incidental miscellaneous materials in the specimens metabolized was very much alike. It seems generally recognized now that there is not such a close correlation between the rate of growth and the metabolism.

METABOLISM OF BUTTER YELLOW RAT LIVER CANCERS.

DEAN BURK, OTTO K. BEHRENS, and KANEMATSU SUGIURA. (Cornell University Medical College, New York, N. Y., National Cancer Institute, Bethesda, Md., and Memorial Hospital, New York, N. Y.)

The rapidly growing interest in hepatic tumors induced in rats by the feeding of dimethylaminoazobenzene (butter yellow) makes it desirable to study the question of whether such tumors do or do not possess a metabolism characteristic of other malignant neoplasms. If they do, their course of development from normal liver offers an excellent opportunity to determine the stage at which the characteristic metabolism begins in relation to the appearance of gross tumor. Table I presents representative data obtained in an extensive series of experiments to be reported in detail elsewhere.

The table shows that the three common characteristics of malignant tumor metabolism; namely, a high anaerobic and a considerable aerobic lactic acid formation ($Q_A^{N_2}$ and $Q_A^{O_2}$, as cu. mm./mgm. initial dry weight/hr.) and a respiratory quotient (R.Q.) definitely less than unity,

TABLE I: THE METABOLISM OF "BUTTER YELLOW" RAT LIVERS, TUMORS,[1] AND REGENERATING LIVER

Material	$Q_A^{O_2}$	Q_{CO_2}	Q_{O_2}	R.Q.	$Q_A^{N_2}$
Normal (no B. Y.)	1.6	3.9	6.0	0.68	1.0
"Normal"—yeast protected	1.6	4.8	4.9	0.98	2.1
"Normal" lobe, adjacent to tumor lobe	1.3	6.2	7.7	0.82	2.3
Cirrhotic liver	1.5	5.4	6.5	0.82	3.1
Necrotic tumor	3.9	3.3	4.2	0.77	6.0
Transplant [2]	2.1	3.1	4.7	0.66	7.7
Metastasis	2.5	4.3	5.0	0.83	8.7
Cholangioma-hepatoma	3.3	6.2	7.3	0.84	10.0
Hepatoma	6.0	5.2	6.4	0.87	12.1
Regenerating liver [3] (Average)	0-1.5	..	..	0.70	0-2.0

[1] Butter yellow fed 150-200 days. Q values based on initial dry weights. (Values would be 50-100% greater if based on final dry weights at end of manometric run.)

[2] 33rd generation, subcutaneously transplanted, liver carcinoma 1 in dilute brown strain. Mouse obtained from Dr. H. B. Andervont through Dr. P. M. West, June 7, 1941; liver of same animal: $Q_A^{N_2} = 0.8$; $Q_A = 1.0$.

[3] 20 to 600 day rats (20 to 400 gm. body weight); regeneration 1 to 10 days, after original 65% hepatectomy (left and median lobes extirpated).

are possessed by the butter yellow hepatomas and cholangiomas, and also by corresponding metastatic and necrotic material. There is a definite, but relatively small anaerobic lactic acid formation in precancerous (cirrhotic) liver as compared to normal liver. There is also a slight increase in "normal" lobes adjacent to tumor-bearing lobes, and in livers of rats protected by yeast feeding. Aerobic glycolysis ($Q_A^{O_2}$), however, increases abruptly over the normal liver value essentially at the onset of gross tumor formation. The respiration (Q_{O_2}) does not change significantly. Results in good part similar to the foregoing have been reported by Japanese and English workers.

In general, the anaerobic and aerobic lactic acid formations in the tumors induced by butter yellow are intermediate in extent between those of the rat, human, and chicken tumors studied by Warburg, on the one hand, and those of the mouse tumors studied later by Murphy and Hawkins and by Crabtree and Cramer. There is a definite aerobic glycolytic mechanism, such as scarcely exists in some mouse tumors, but the anaerobic glycolysis is not as great as that reported for most rat, human, and chicken malignant tumors. Compared to that of the origi-

nal liver tissue, however, where the anaerobic lactic acid formation is almost negligible, the increase in the hepatic tumor is relatively very marked.

The question of whether the increased lactic acid formation in hepatic tumors—or tumors generally—is *necessarily* an expression of increased growth involved, as commonly believed, has been largely resolved in experiments to be detailed elsewhere and carried out in cooperation with Dr. J. Saxton, J. Blanchard, C. Povolny, and J. Norris. Rapidly growing embryonic rat liver showed marked glycolysis as compared to adult liver, but this was closely correlated with, and was probably accounted for by, the enormous number of erythroid and polynuclear cells; the oxygen consumption was normal. Regenerating liver, with a growth rate equal to that of the hepatic or other tumors generally, or of even most growing tissues, showed no increase in metabolic values compared to normal liver, and in particular no increase in lactic acid formation. Growth, therefore, whether of normal or of malignant animal tissue, does not necessarily require glycolysis, and may be maintained at a high level on an essentially aerobic nonfermentative metabolism.

Discussion

O. ROSENTHAL (Philadelphia, Penn.): The metabolism of rat liver differs from that of malignant tumors quantitatively and qualitatively. The rate of glycolysis depends on the state of nutrition of the animal. In most of the malignant tumors there is either no succinic-dehydrogenase or at least its activity is very low. Have you made observations on fructose and succinate metabolism in malignant tumors of the liver and in normal parts of the same livers?

D. BURK: We have not made these comparisons, which would be most pertinent and interesting.

J. S. GREENSTEIN (Bethesda, Md.): In reference to Dr. Rosenthal's question we have some data on xanthine oxidation. We found this activity much lower in liver tumor than in normal liver tissue.

K. STERN (New Haven, Conn.): What is the definition of the term "malignant tumor metabolism"?

D. BURK: Briefly, this type of metabolism is characterized by high anaerobic glycolysis and an intermediate R.Q. (0.75 to 0.9, ±). These properties are characteristic of at least 90 per cent of all malignant tumors. A very few nontumor tissues may have this type of metabolism. If need be, further differentiation may be made on the basis of magnitude of respiration and aerobic glycolysis, or of certain derived quotients (absolute and per cent Pasteur effect, fermentation excess, ratio of glycolysis to respiration, etc.)

K. STERN: In accordance with the brief definition given, the metabolism of young embryonic tissue would be classified as malignant, except for the respiratory quotient, where the value is still questionable. I would emphasize that if there is a characteristic malignant metabolism the distinctive feature consists of the ratio of aerobic to anaerobic glycolysis. The point of aerobic glycolysis seems to have been neglected in the speaker's discussion.

D. BURK: The matter of aerobic glycolysis does not appear to be as important today as it did originally some fifteen or so years ago. As Dr. Murphy found many years ago, aerobic glycolysis scarcely occurs in a number of highly malignant tumors of the mouse. From my point of view, aerobic glycolysis is largely, even if not entirely, a consequence of how much anaerobic glycolysis goes on in relation to how much oxygen consumption occurs in the particular tissue under examination.

E. S. G. BARRON (Chicago, Ill.): Apparently Dr. Burk has changed his opinion since he spoke at the Cold Spring Harbor symposium on the metabolism of tumors. I do not agree with the dismissal of the significance of aerobic glycolysis, as it is still important to study carbohydrate breakdown in the presence as well as in the absence of oxygen. The slowly rising value of lactic acid formation in the presence of oxygen exhibited by butter yellow carcinoma shows that aerobic lactic acid formation has to be taken into consideration in studies of the metabolism of tumors.

D. BURK: The latter statement is certainly correct, but the aerobic glycolysis should still be used as a subsidiary characteristic. In order of importance, anaerobic glycolysis is first, the R.Q. second, and aerobic glycolysis (together with magnitude of respiration) third. I believe this view is indeed equivalent to my Cold Spring Harbor symposium analysis.

PHOSPHATASE METABOLISM OF NORMAL AND TUMOR TISSUES IN CULTURE. A. M. BRUES and W. E. COHN. (Collis P. Huntington Memorial Hospital, Boston, Mass.)

The phosphate uptake and metabolism of various growing and surviving cells was studied, using the readioactive isotope of phosphorus P^{32}). With the roller bottle technic, it is possible to estimate the cell P^{32} independently of that in the medium without disturbing the cultures. Increase in cell P^{32} may represent: (a) exchange of phosphate across the cell surface; (b) turnover of phosphate between various compounds within the cell; (c) growth of cells. Growth of cells can also be estimated by uptake of total phosphorus from the medium. The rate and amount of P^{32} uptake by cells correlates strikingly with growth, and no striking differences in this respect were seen between various normal and malignant cells. A critique of existing methods of estimating growth in tissue cultures was presented.

Discussion

F. LIPMAN (New York, N. Y.): Is there any difference between the metabolism of fat and the turnover of the phosphates when glucose is replaced by other substances?

A. M. BRUES: Our cultures have been grown only in media containing glucose and peptone. We do not yet have data concerning fat metabolism. The cells growing in this medium contain very little fat.

THE EFFECT OF AVAILABILITY OF DIETARY ORGANIC SULFUR ON THE REACTION OF DILUTE BROWN MICE TO METHYLCHOLANTHRENE. G. B. MIDER and J. WHITE. (National Cancer Institute, Bethesda, Md.)

Young mice of the dilute brown strain were fed a synthetic diet containing adequate amounts of all known sub-

stances necessary for growth excepting the sulfur-containing amino acids, cystine and methionine. The mice failed to gain weight. When the diet was supplemented with 0.5 per cent l-cystine, weight increment proceeded.

Dilute brown mice that ingested the high and low cystine diets were painted on alternate days with a 0.2 per cent solution of methylcholanthrene in ethyl ether. Ninety per cent of the mice that ate the high cystine diet and 19 per cent of the mice that were fed the low cystine diet developed leukemia. Ninety per cent of the mice that ingested the low cystine diet had degenerative and sclerotic lesions of the aorta and other large arteries at necropsy. The vascular changes consisted principally in degeneration and calcification of the media, with plaque formation, and thickening of the adventitia.

The reduction in the leukemia incidence by a deficiency in dietary cystine may be more apparent than real since most of the mice with vascular lesions died before the others developed leukemia. It is suggested that the vascular damage was a manifestation of the toxicity of methylcholanthrene probably not related to its carcinogenic properties.

Discussion

W. C. HUEPER (New York, N. Y.): In this connection the work of McCay is to be recalled. He observed that a severe type of calcifying sclerosis developed in young rats fed a diet barely adequate for the maintenance of life. The vascular lesions observed may therefore represent the result of a nutritional inadequacy aggravated by the detoxication of methylcholanthrene.

THE PREVENTION OF MALIGNANCY OF THE VULVA. FRED J. TAUSSIG. (Barnard Free Skin and Cancer Hospital, St. Louis, Mo.)

This etiologic study of 161 cases disclosed a number of pre-existing lesions which tended toward the development of malignancy. The prompt removal of such lesions may reduce the incidence of cancer of the vulva.

To be published in *Cancer Research*.

THE EFFECT OF ANDROGENS AND ESTROGENS ON SPONTANEOUS BENIGN MAMMARY TUMORS. JACOB HEIMAN. (Department of Cancer Research, Columbia University, New York, N. Y.)

The effect of exogenous hormones on transplanted mammary fibroadenoma in the rat has been published (*Am. J. Cancer*, **39**:172-177, 178-184. 1940). This report is an account of a similar study on spontaneous benign mammary tumors occurring in white rats. Ninety-seven rats with spontaneous tumors were used in the experiments. All animals were observed until death, the period of observation extending from one to two years. Auto- and homotransplants of the tumors were made. Series of animals were treated with injections of estradiol benzoate, or testosterone propionate, or with combinations of these hormones.

Conclusions drawn from the observations follow: 1. with minor variations, autotransplants, for two to five tumor generations, retain the architectural identity of the original tumor; 2. homotransplants of adenofibroma in a rat freed from spontaneous fibroma became a cellular fibroma; 3. homotransplants of fibroma in a rat freed from adenofibroma remained a fibroma, unless the rat was estrinized; 4. estrogens stimulate the epithelial components of early spontaneous auto- or homotransplanted adenofibroma, producing adenoma or cystadenoma; 5. androgens inhibit the epithelial components; 6. estrogens administered with androgens overcome the inhibitory action of the androgens and exert a stimulating effect on growing specific epithelium; 7. connective tissue elements of the tumor are not directly affected by estrogens and are moderately inhibited by androgens.

Discussion

W. U. GARDNER (New Haven, Conn.): As far as antagonistic effects are concerned the estrogens are much more potent than the androgens in the normal reactions elicited by these compounds. The same ratio of physiologic activity between the estrogens and androgens is shown in the benign tumors.

H. J. BAGG (New York, N. Y.): Were any spontaneous tumors found in the male rats and, if so, were these in animals of the Wistar stock?

J. HEIMAN: Cystic degeneration of the testis occurred in one rat, with a mammary adenoma, and two rats had fibromas. One animal was of the Wistar stock.

H. J. BAGG: By transplanting ovarian tissue into male rats we succeeded in stimulating the mammary gland to active growth.

W. CRAMER (St. Louis, Mo.): From the results of experiments which could not be finished I received the impression that when estrogens and androgens are administered in certain quantitative combinations, the effect of the estrogen is reinforced by the androgen. If the mammary glands of males of a high cancer strain are caused to proliferate by injection of estrogens mammary tumors are produced more easily in the males than in the females. This strengthens the idea that a combination of estrogen and androgen may actually be more powerful than estrogen alone.

J. HEIMAN: Examinations were made of the pituitary and adrenal glands of all animals. Adenomas of these glands occurred occasionally.

RESULTS OF A STUDY OF LIVING BLOOD OF CANCER PATIENTS. GEORGE A. WYETH. (Hanover, N. H.)

Two small structures were found in the fresh blood of patients with cancer when the blood prepared in a specified manner was examined by dark field illumination. The first body was designated WIG. This body, measuring 0.5×1.0 μ in diameter, was seen in coccoid, diplococcoid, triplococcoid, and tetracoccoid forms. It was always highly refractile, of frosty appearance, and red or white. It had a wiggling jerky motion in addition to Brownian movement. This body is constantly present in blood of cancer patients and to some extent in so-called normal blood. The second body was filamentous. It differed from pseudospirochetes and filaments seen in normal blood. These filamentous bodies, which apparently have not been de-

scribed previously, are delicate, faint structures, which progress in the drop of blood by a motion resembling that of the earthworm, contracting and elongating. Transverse division is occasionally seen.

RESULTS OF SURGERY IN CANCER OF THE STOMACH. H. WILLY MEYER. (New York, N. Y.)

A patient with extensive linitis plastica of the stomach and lower esophagus was successfully treated by total gastrectomy and partial esophagectomy. The operation, apparently the first of its kind, demonstrated the possibility of extensive resection by the combined abdominal and transthoracic routes and the re-establishment of continuity of the alimentary tube by intrathoracic esophagojejunostomy.

EFFECTS OF ESTROGEN ON THE GROWTH AND STRUCTURE OF A TRANSPLANTABLE CARCINOMA OF THE RAT BREAST. MILTON J. EISEN. (Department of Cancer Research, Columbia University, New York, N. Y.)

Published in *Cancer Research*, 1:457-464. 1941; abstract p. 506.

THE INFLUENCE OF SEX ON IRON ASSIMILATION IN THE RAT. S. W. KLETZIEN. (New York State Institute for the Study of Malignant Diseases, Buffalo, N. Y.)

Various published reports on the influence of sex on iron assimilation convey the impression that the female organism more efficiently assimilates iron than the male. Our data based on iron analyses of the total blood, liver, carcass, and spleen do not bear out this impression. We have found as have others that the female responds more quickly with a higher concentration of hemoglobin and exhibits evidence of greater liver stores of iron than the male but presumably these result from the lesser requirements for growth in contrast to those of the male. The male puts out a larger volume of blood of a lower hemoglobin content and builds into its soft tissues more iron on the other hand, thereby offsetting any apparent gains in the female and actually exceeding them in total iron assimilated.

Carrying our studies further we have found that ovariectomy increases iron assimilation as does castration. Such procedures nullify any differences in the pattern of iron distribution and bring about comparable response in the two sexes. Pregnant females as compared with virgin litter mate controls and males reveal an increased capacity to assimilate iron on similar iron, copper, and basal diet intakes. This in itself is, however, not sufficient to maintain prepregnancy stores of iron. The iron assimilation of the pregnant female thus simulates that of the rapidly growing male.

Discussion

G. M. SMITH (New Haven, Conn.): What was the distribution of iron in the lymph nodes?

S. W. KLETZIEN: Our studies did not include analyses of lymph nodes.

MAMMARY GLAND TUMORS IN THE MALE RAT ASSOCIATED WITH CASTRATION AND OVARIAN TRANSPLANTATION. H. J. BAGG. (Memorial Hospital, New York, N. Y.)

Abstract not available.

PATHOLOGICAL FEATURES OF TUMORS OF STRAIN A MICE INDUCED BY ULTRAVIOLET RADIATION. H. G. GRADY, H. F. BLUM, and J. S. KIRBY-SMITH. (National Cancer Institute, Bethesda, Md.)

Male mice of strain A subjected to measured amounts of ultraviolet radiation developed tumors of the external ear in the great majority of cases and in a few instances tumors of the eye. With the dosages employed spindle cell sarcoma was by far the most frequent type of tumor encountered although carcinoma was also observed. The histologic character of these tumors and their sites of origin were described and discussed.

Discussion

G. M. SMITH (New Haven, Conn.): Were any changes found in the blood vessels of the skin?

H. G. GRADY: The earliest reaction to ultraviolet radiation is a vascular response. This is quickly followed by a rather rapid epidermal hyperplasia. The rate of epidermal hyperplasia, however, seems definitely to slow down while the inflammatory reaction in the subepidermal tissue continues.

In this series of animals sarcomas were not only considerably more frequent but also arose at an earlier date than the carcinomas.

W. CRAMER (St. Louis, Mo.): Was there any epilation of the mice as a result of the radiation?

In some of my specimens and in some of the specimens shown by Dr. Grady there is the condition of squamous epithelium developing into spindle cells. This makes it difficult to be certain whether these so-called sarcomata, found under these conditions, may not be spindle cell carcinomata. Such spindle cell carcinomata have been previously described. It is sometimes impossible to decide whether they are sarcomata or carcinomata.

H. G. GRADY: The degree of epilation is relatively small and is most marked about the snout. How much of this is due to the radiation and how much to the movement of the mice in the metal holders is impossible to determine. With larger dosages of radiation epilation is definite.

It is a fact that the so-called spindle cell carcinoma is hard to distinguish from the sarcoma. Nevertheless there are definite differences. With hematoxylin and eosin, there is relatively less cytoplasm and more nucleus. With reticulum stains there is shown an extremely close, fine network in the sarcomas which is not present in the carcinomas. In sections stained with phosphotungstic acid hematoxylin it was usually easy to demonstrate fibrils in the sarcomatous growths.

THE PRODUCTION OF TUMORS BY TRANSPLANTATION OF NORMALLY APPEARING LIVER CELLS FROM ANIMALS PREVIOUSLY INJECTED WITH METHYLCHOLANTHRENE. W. A. SELLE, P. BRINDLEY, and J. W. SPIES. (University of Texas School of Medicine, Galveston, Texas.)

Published in *Cancer Research,* 1:618-619. 1941; abstract p. 669.

Discussion

H. L. STEWART (Bethesda, Md.): The sarcomas induced in the subcutaneous tissues by methylcholanthrene rarely metastasize to the liver. The experiments just reported would be more convincing, however, if the liver tissue, before injection, were subjected to heat sufficient to kill any living cells. This should not alter the methylcholanthrene or any other carcinogenic agent of that nature which might be present.

M. B. SHIMKIN (Bethesda, Md.): Evidence from our unpublished observations would indicate that probably methylcholanthrene was present in these livers and was perhaps responsible for the induction of tumors when the tissue was transplanted.

The negative results with C57 mice are difficult to understand, unless the livers of C3H mice were transplanted into C57 mice.

W. A. SELLE: We have used cell-free filtrates of the liver, but have not heated the liver hash. We thought that in view of the fact that these tumors developed in 3 weeks the methylcholanthrene factor could be ruled out. However, in some instances carcinogens have produced tumors in that short period.

CYTOLOGICAL AND CULTURAL OBSERVATIONS ON TRANSPLANTABLE RAT SARCOMATA PRODUCED BY THE INOCULATION OF ALTERED NORMAL CELLS MAINTAINED IN CONTINUOUS CULTURE. GEORGE O. GEY. (Johns Hopkins University School of Medicine, Baltimore, Md.)

This paper, illustrated by motion pictures, was a chronological report of observations made on continuous cultures of normal mesenchyme cells and of the occurrence of cytological changes which rendered these cells potentially malignant as demonstrated by the production of transplantable sarcomas of different cytological types.

Discussion

M. J. EISEN (New York, N. Y.): The circumstances under which these normal cells in tissue culture became malignant was not made clear.

G. O. GEY: We have no special knowledge of the circumstances which rendered these strains of rat mesenchyme a tumor-producing strain.

Certain factors should be considered as probable tumor cell-producing agents. One of these factors concerns the medium in which the cells were grown. The medium which we used on these cultures contained about 60 per cent human (placental) cord serum in addition to chicken plasma and bovine embryo extract. Since the cord serum has a rather high concentration of estrogenic substances one is justified in assuming a possible role of these substances as tumor-inducing agents. However, we have not at any time before this observed the occurrence of a permanent alteration in any one of the many strains of cells that we have carried in a period of almost 20 years of experience with continuous cultures.

The cultures which became permanently altered were given no specific treatment that was not also given to many other strains of normal and tumor cells carried at the same time and in the same media.

One other factor should be mentioned as of possible significance. The incubator in which all of our continuous cultures were placed was several meters from a heavily shielded lead radiation chamber. Careful measurement showed that for a period of several months all of our pure strain cultures, about 40 strains in all, were exposed to about 200 times the natural earth radiation or the equivalent of about 0.25 r of gamma radiation. There is a very slight possibility that this stray gamma radiation produced the above alteration, but I doubt it very much. In order to get some idea of the effects of continuous gamma radiation on rat mesenchyme cells of several types we exposed a number of strains to doses many times that to which the altered strains and other strains were accidentally exposed. Such radiation produced no permanent alterations of cells in continuous cultures. Cultures injured by irradiation and inoculated produced no tumors.

TUESDAY AFTERNOON SESSION—APRIL 15

DR. BURTON T. SIMPSON, PRESIDENT, PRESIDING

SEX HORMONE EXCRETION RATES IN HIGH AND LOW TUMOR STRAINS OF MICE. J. C. AUB, D. KARNOFSKY, and L. E. TOWNE. (Collis P. Huntington Memorial Hospital, Boston, Mass.)

The relation of the sex hormones to the incidence of breast tumors in susceptible mice is well known. Therefore, the possibility that the high incidence of spontaneous mammary tumors in the C3H and the low incidence of tumors in another strain (C57) was due to differences in normal sex hormone metabolism appears worth considering. Although indirect, one of the most valuable methods of getting an index of hormone metabolism is the measurement of the urinary excretion of these substances.

Three groups of 24 mice in each; 1. virgin C3H, 2. once-pregnant C3H, and 3. virgin C57, 5 months of age and weighing 20 to 25 gm., were used. These were kept in metabolism cages over a period of 5 months, urines were collected daily, and pooled 2-week specimens from each group were assayed. Estrogens were determined biologically and 17-ketosteroids colorimetrically.

The average estrogen excretion per mouse per day was 0.05 to 0.10 I.U., and 17-ketosteroids 0.04 to 0.08 mgm. per mouse per day. These showed suggestive variation between the strains, but no significant differences were noted and no marked changes occurred following pregnancy, or with increase in age from 5 to 10 months, or with the appearance of tumors. Thus, while differences may exist they are not of a magnitude that can be demonstrated by our technic.

THE EFFECT OF ESTROGEN ON THE INCIDENCE OF MAMMARY AND PITUITARY TUMORS IN HYBRID MICE. W. U. GARDNER. (Yale University School of Medicine, New Haven, Conn.)

Published in *Cancer Research,* 1:345-358. 1941; abstract p. 424.

CANCER OF THE CERVIX OF THE UTERUS IN HYBRID MICE FOLLOWING LONG-CONTINUED ADMINISTRATION OF ESTROGEN. EDGAR ALLEN (by invitation) and W. U. GARDNER. (Yale University School of Medicine, New Haven, Conn.)

Published in *Cancer Research,* 1:359-366. 1941; abstract p. 423.

Discussion

W. C. MacCARTY, SR. (Rochester, Minn.): How does the carcinogenic dose compare with the human therapeutic dose? I ask this question because I am just beginning to see enormous cysts in the cervices of women, which I did not formerly see. In some cases by looking at the uterus containing these cysts I can almost tell whether or not the patients have been treated with these estrogenic substances.

How do you explain the present higher percentage of positive results as compared with lower percentages reported formerly?

E. ALLEN: I cannot even guess at the dose of estrogen that might produce cervical cancer in women. The time factor would probably be equally important. For instance, in 1931, Dr. Oberholtzer and I began attempts to produce cervical cancer in monkeys by chronic estrogenic stimulation, and these experiments have been continued. In all this time no cancerous lesion has appeared, although some atypical growths, previously considered precancerous, have occurred.

Several factors account for the high incidence of cervical cancer in these hybrid mice. These mice, selectively bred by Dr. Gardner, are resistant to mammary cancer and tolerate well prolonged treatment with estrogens. In comparison with the earlier series of mice which were killed by mammary cancer and did not tolerate chronic estrogenic treatments well, the animals used in these experiments survived estrogenic treatment for a year or longer and hence gave oportunity for the development of cervical cancer.

FURTHER STUDIES ON THE EFFECT OF THE MILK-INFLUENCE UPON THE PRODUCTION OF SPONTANEOUS AND INDUCED TUMORS OF THE BREAST. J. J. BITTNER. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

Read by title.

THE EFFECT OF THE MILK FACTOR CONCENTRATION ON THE DEVELOPMENT OF SPONTANEOUS TUMORS IN HYBRIDS DERIVED FROM RECIPROCAL CROSS OF TWO HIGH CANCER STRAINS OF MICE. STANLEY G. WARNER. (New York State Institute for the Study of Malignant Disease, Buffalo, N. Y.)

Mice of this inbred stock arrange themselves on a graduated scale of cancer incidence because of (a) the concentration of the milk stimulus which they transmit, and (b) the resistance to the stimulus. By the methods presented it is possible to measure roughly the difference in the amount of cancer which these concentrations of the extrachromosomal stimulus will produce. It is found that the A stock factor will produce approximately three times as much cancer as the Marsh stock factor when both are acting upon the same physiological constitution.

STUDIES ON THE EFFECT OF FOSTER NURSING AND ITS RELATION TO THE DEVELOPMENT OF MAMMARY CARCINOMA IN THE MOUSE. W. S. MURRAY. (New York State Institute for the Study of Malignant Diseases, Buffalo, N. Y.)

It was the purpose of this paper to demonstrate, by reports of observations and analyses of data, that the occurrence of mammary cancer in mice may be reasonably explained upon the basis of the strength of the extrachromosomal stimulus which the mice receive and the physiological resistance of the animals of the various stocks and crosses to this stimulus.

Discussion

C. C. LITTLE (Bar Harbor, Me.): The method Dr. Murray is using is a very interesting attempt to work out a quantitative determination of the concentration of the milk influence. While it will be necessary to do some further experimentation to eliminate the effect of genes, the results he has obtained and the hitherto unexplained diminution of tumors in hybrid generations of backcrosses fit very well together in a preliminary way.

J. G. KIDD (New York, N. Y.): I did not clearly understand what Dr. Murray meant by resistance to the extrachromosomal stimulus.

W. S. MURRAY: It has been shown quite conclusively that of the inbred stocks, the C57 black strain is one of the most resistant to the development of mammary tumors and that the dilute browns are among the least resistant. Mice of these stocks are quite different physiologically. If we think of their physiologies as being determined by their chromosomal constitutions, hybrids between the C57 blacks and dilute browns should be physiologically different from either parent stock. If these three physiologies are all acted upon by the dilute brown extrachromosomal or milk stimulus, varying degrees of tumor development are found. The blacks are the most resistant, the dilute browns the least, and the hybrids are intermediate between these extremes.

GENETIC ANALYSIS OF THE INDUCTION OF TUMORS BY METHYLCHOLANTHRENE. II. THE INFLUENCE OF SPINDLE CELL SARCOMA AND OF CARCINOMA OF THE SKIN UPON EACH OTHER. L. C. STRONG. (Yale University School of Medicine, New Haven, Conn.)

Published in *Cancer Research,* 1:572-574; abstract p. 584.

Discussion

M. B. SHIMKIN (Bethesda, Md.): In using inbred lines of mice do you get only one type of tumor with this technic?

L. C. STRONG: No, you get variations.

M. B. SHIMKIN: How is that explained?

L. C. STRONG: The whole investigation of that problem must be put on a larger basis. Data from more experiments with hybrids must be obtained before the final genetical analysis may be made.

M. B. SHIMKIN: It is difficult to see how the fact of contact with this carcinogen can be eliminated, because contact of carcinogen with various tissues must be taken into consideration.

L. C. STRONG: Contact between carcinogen and tissue is important for certain types of tumors, but the distributional factor is controlled by the constitution of the host. By selection through hybridization I have been able to obtain sublines in which only specific types of tumors occur under controlled conditions.

J. FURTH (New York, N. Y.): Could Dr. Strong tell us something more about the most important and original part of this contribution; namely, the production of intestinal polyps—their incidence, and location, and whether any one of them showed a tendency to become malignant?

L. C. STRONG: The analysis of specific types of tumors aside from spindle cell sarcoma and carcinoma of the skin must wait for some future time. Malignant intestinal polyps do occur after the subcutaneous injection of methylcholanthrene. They take a long time to develop. The first one I observed occurred in a mouse 471 days after the injection of the carcinogen.

M. J. EISEN (New York, N. Y.): It is possible that the squamous cell cancers were not derived from the skin but from the mammary ducts. In a series of 1,000 methylcholanthrene-induced tumors reported from the Department of Cancer Research at Columbia we found all grades of involvement of the adjacent mammary epithelium around nodules of the methylcholanthrene injected in liquid paraffin which solidified under the skin. The incidence of breast tumors was about 4 per cent. Most of these were mixed with spindle cell sarcoma. The breast cancers were either squamous cell cancer or adenocarcinoma, but there was no indication that these squamous cell formations and subsequent squamous cell cancers developed from the skin. They developed, in our experience, from the mammary ducts.

In ten inbred rat strains there was a random distribution of various types of tumors, including mammary tumors. In agreement with Dr. Shimkin's opinion we regarded the results as indicating that the distribution of types of tumors depended upon the chance contact of a given cell with the specific carcinogenic agent.

C. C. LITTLE (Bar Harbor, Me.): It is possible, as Dr. Strong believes, to have a genetic basis for characteristics of inbred strains, and still to have a variation in the appearance of the character which is fixed within a strain but is not necessarily dependent upon localizable genes. Evidence for this is provided by the results of investigations of Wright upon morphological abnormalities of guinea pigs and of Johansen and others on beans.

L. C. STRONG: In answer to Dr. Eisen's comments, there was no sex difference in the occurrence of these squamous cell cancers. Males as well as females give rise to these tumors. I feel sure, in addition, that methylcholanthrene does not stimulate mammary tissue, and that these were not duct tumors. I have serial sections showing the origin of these tumors from the skin.

M. J. EISEN: In our experiments methylcholanthrene stimulated the mammary duct. In both male and female animals mammary ducts surrounding nodules of methylcholanthrene have been frequently the site of hyperplastic changes. Squamous cell metaplasia commonly occurred in these hyperplastic zones, and at times squamous cell cancer developed as a result of the continued action of the carcinogen.

L. C. STRONG: Some of these tumors arise from hair follicles in mice.

THE INFLUENCE OF HYBRIDIZATION UPON THE SUSCEPTIBILITY OF MICE TO TRANSPLANTABLE AND SPONTANEOUS TUMORS. H. B. ANDERVONT. (National Cancer Institute, Bethesda, Md.)

When sarcoma 37 was implanted cutaneously into strain Y mice it grew progressively and killed the animals but when implanted cutaneously into mice of strains I or L it receded spontaneously in practically every animal. The response of hybrid mice derived from strains I and Y indicated that susceptibility to progressive growth of the tumor was inherited in a dominant manner and suggested that a single factor was involved. When hybrids derived from strains I and L were inoculated the tumor grew progressively in most of the F1 hybrids which suggested a summation of genetic factors from two resistant strains resulting in highly susceptible hybrids. Backcross generations obtained from strains L and I produced results indicating the presence of a number of genetic factors.

When female mice of low mammary cancer strains, C57 black and I, were foster nursed by high mammary cancer strain C3H females only from 15 to 20 per cent developed mammary carcinoma. Hybrids derived from strains C57 black and I were foster nursed by strain C3H females and were found to be highly susceptible to the development of mammary tumors. This finding also suggested a summation of genetic factors resulting in susceptible hybrids.

THE ROLE OF HEREDITY AND EXTRINSIC FACTORS IN THE CAUSATION OF LEUKEMIA. J. FURTH and R. K. COLE. (Cornell University College of Medicine, New York, N. Y.)

Data are presented on the incidence of leukemia in a high-leukemia stock (AK) and low-leukemia stock (Rf) and their hybrids. The data are interpreted by assuming that multiple genetic factors determine the occurrence of this disease. There is in addition a maternal influence, and the disease occurs more often among female than male mice. The mode of inheritance of leukemia varies with different stocks.

Leukemic cells from spontaneous and induced leukemia differ markedly. The former resemble genetically the leukemic ancestor. The latter vary greatly among themselves; they may resemble the hybrid cell or differ from both parents and hybrids. This suggests the possibility that cells from induced leukemias are mutants.

ON THE METHOD OF INHERITANCE OF SUSCEPTIBILITY TO LUNG TUMORS. CLARA J. LYNCH. (Rockefeller Institute for Medical Research, New York, N. Y.)

Comparisons are made of three inbred strains of mice (Swiss-8, Bagg, and 1194) observed (a) under ordinary laboratory conditions, (b) after cutaneous tarring, and (c) after intraperitoneal injections of 0.5 minimum of a 0.4 per cent solution of 1,2,5,6-dibenzanthracene into mice 2 to 4 days old.

The number of tumor nodules per mouse used as a criterion of susceptibility brings out greater differences than when only the percentage of tumors is noted. The groups, under the various classifications, fall into a graded series showing the effects of both hereditary and environmental differences.

The results indicate that susceptibility is not a single genetic entity but that various degrees of susceptibility may be inherited.

A RELATIONSHIP BETWEEN SUSCEPTIBILITY TO PULMONARY TUMORS AND KNOWN GENES IN MICE. W. E. HESTON. (National Cancer Institute, Bethesda, Md.)

During the past three years experiments have been conducted in an attempt to determine whether or not susceptibility to pulmonary tumors, induced by methylcholanthrene, is associated with certain chromosomes of the mouse. This has been done by testing for linkage between susceptibility and known genes which are carried on the different chromosomes. Data which already have been reported failed to give evidence of linkage to any of the five coat color genes, brown, agouti, dilution, leaden, and piebald.

The purpose of this paper is to present data which show that susceptibility to pulmonary tumors is associated with the chromosome marked by the shaker-2 and waved-2 genes and also strongly suggest an association with the one carrying the flexed tail gene.

The two strains of mice used in testing for linkage to these three recessive genes were strain A which shows a high degree of susceptibility to pulmonary tumors and strain W which carries the three factors in the homozygous condition. The strain A mice were derived from animals obtained from Dr. Bittner's strain A colony at the Roscoe B. Jackson Memorial Laboratory in 1938, while the strain W was developed by Dr. Snell at the same laboratory.

In conclusion it can be stated that a relationship between susceptibility to induced pulmonary tumors and certain known genes has been found. In the backcross generation here described mice displaying the shaker-2 and waved-2 characters were less susceptible than the normal mice and also those with flexed tails tended to be less susceptible than those with normal tails.

Discussion

C. C. LITTLE (Bar Harbor, Me.): It seems to me that Dr. Heston's paper presented an extremely important and significant piece of work. For the first time clear evidence has been given of the association between individual chromosomes and the genetic basis of tumor formation. He has conclusively shown a chromosomal influence of a definite genetic nature which can be used in the study of linkages.

INCIDENCE OF LUNG TUMORS IN FIVE RELATED STRAINS OF MICE AND THEIR HYBRID DERIVATIVES. MAUD SLYE. (University of Chicago, Chicago, Ill.)

Among 1,666 mice of 5 related strains and their hybrids that lived into the age to be tested for lung carcinoma, there was 23.2 per cent of spontaneous lung carcinoma and 3.5 per cent of benign tumor of the lung. Lung carcinoma occurred in 20.6 per cent of the females and 28.9 per cent of the males. Thus lung carcinoma was slightly more frequent among the males. The benign tumors did not behave genetically like malignant tumors.

These were long-lived strains and the highest incidence of lung tumor was consistently in the 20 to 23 month age group. The oldest mice in every group were the mice without lung tumor. Thus lung tumor is not necessarily related to senescence but rather to an inherited time factor within which susceptible lungs lose their resistance to the irritation factor. The percentages of lung tumor were similar among the 5 lines and their hybrids. Thus when the lung carcinoma percentages are known in both sides of a hybrid cross, the approximate lung tumor percentage in the hybrids can be predicted in age-tested mice.

In 423 mice selected in line 7 from a closely inbred genetic group derived from two litter sisters, both with lung carcinoma of high malignancy and each mated with a litter brother, there was a rapid decrease in the lung tumor incidence from 42.9 per cent in F1 to 0 per cent in F6 (the number of age-tested mice in F6 was small).

There were 34 crosses in this group. Fifteen crosses were between one parent with lung carcinoma and one parent without lung tumor (but derived from lung tumor). Nineteen crosses were between parents without lung tumor (but derived from lung tumor). One parent in some of these crosses was not age-tested.

In the crosses where one parent had lung tumor, the F1 gave two consistent results. Lung carcinoma occurred:

1. In 1 out of 2.4 among 85 age-tested mice from 6 crosses.
2. In 1 out of 4.6 among 111 age-tested mice from 9 crosses.

The F1 from parents without lung tumor gave three consistent results. Lung carcinoma occurred:

1. In 1 out of 2.8 among 69 age-tested mice from 4 crosses.
2. In 1 out of 3.9 among 59 age-tested mice from 5 crosses.
3. In 1 out of 8.2 among 99 age-tested mice from 10 crosses.

These results suggest a minimum of two recessive factors, one for type and one for site, and also an age deter-

minant (a possible third factor) as expressing the degree of resistance to the irritation factor.

HYPERAUXINY IN CROWN GALL. GEORGE K. K. LINK (by invitation) and VIRGINIA EGGERS (by invitation). (University of Chicago, Chicago, Ill.)

Abstract not available.

Discussion

M. J. SHEAR (Bethesda, Md.): In connection with these experiments of Dr. Link, it may be of interest to mention that three of the hetero-auxins when administered subcutaneously to mice for a year and a half failed to induce tumors or proliferation of any sort.

GROWTH OF YEASTS IN THE PRESENCE OF CARCINOGENS. W. A. CHILDS (by invitation), JOHN W. SPIES, and J. K. CLINE (by invitation). (University of Texas School of Medicine, Galveston, Texas.)

Abstract not available.

PROPERTIES OF THE FRACTION OF B. PRODIGIOSUS WHICH PRODUCES HEMORRHAGE IN MOUSE SARCOMAS. J. L. HARTWELL (by invitation), M. J. SHEAR, H. KAHLER (by invitation), and F. C. TURNER. (National Cancer Institute, Bethesda, Md.)

Last year Shear and Turner reported the preparation of a concentrate from *B. prodigiosus* filtrate which produced hemorrhage in mouse sarcoma in doses as low as 0.2 cc. of a 1:10,000 dilution. This dose contained 0.002 mgm. of total solids. Since then further observations have been made on various chemical and physical properties of the active fraction.

Tests of the concentrate for protein gave negative results with the biuret and ninhydrin reactions. The dried lyophilized residue, redissolved in water, gave negative tests with the xanthoproteic and Millon's reactions. If this fraction contains any protein it is present to the extent of less than 1 per cent.

The concentrate gave a strongly positive Molisch test for carbohydrate. The Benedict test for reducing sugars was negative as well as the iodine-iodide test for starch, glycogen, and dextrins. Since this material was not dialyzable through cellophane over the pH range 1 to 10 it obviously consisted of high molecular weight components.

On slow drying of the concentrate over $CaCl_2$ at room temperature, a glassy, amorphous residue was obtained which did not redissolve in water; this dried material still contained 11 per cent water. Freezing of active solutions destroyed most of the potency.

Study under the microscope of residues obtained by precipitation and evaporation under the various conditions indicated that the active fraction was not homogeneous, but consisted of a number of constituents.

The material was fractionated by high-speed centrifugation and by electrophoresis and numerous fractions were assayed for hemorrhage-producing potency in mice bearing transplanted sarcoma 37. The ABC mice used were a cross between the A and C57 black strains, and were of three colors. All mice responded similarly to these materials. To determine the potency of a preparation about 200 to 250 mice were used in each experimental assay. The methods of assay were described in detail.

Centrifuging of the concentrates at 4,000 times gravity gave a small amount of sediment, which had negligible activity. The turbid supernatant solution was then centrifuged at 23,000 times gravity for periods up to 10 hours. A jelly-like sediment was obtained which possessed high activity, but considerable protency was still left in the supernatant solution. When this solution was then ultracentrifuged for 3 hours at 160,000 times gravity, some additional active sediment was obtained, but a significant proportion of the activity remained unsedimented. When another aliquot of the concentrate was ultracentrifuged in the same way, but without prior centrifuging at lower speeds, practically all of the activity was removed from the solution, and was present in the sediment. This raised the question as to whether the active agent might not be a substance of lower molecular weight than the bulk of the sedimented material on which it might be adsorbed or bound in some way, and from which it could be dissociated.

The electrophoresis experiments at pH 6.8 gave evidence which also appears to point in the direction of some separation. The turbid material migrated toward the anode, leaving a clear zone in the opposite arm of the U-tube. The migrated, turbid fraction, although highly potent, was usually less active than the original material. The clear zone, although less active, still had significant potency. Thus two active fractions have been obtained with different electrical and other properties. So far no indication of an iso-electric point has been obtained.

These results show that the concentrate contains a number of constituents with different properties. These differences are being studied from the point of view of developing a method for further purification, and identification, of the active agent.

FURTHER STUDIES ON THE EFFECT OF X-RAYS ON A TUMOR OF KNOWN GENETIC CONSTITUTION. S. G. WARNER, M. C. REINHARD, and H. L. GOLTZ. (New York State Institute for the Study of Malignant Diseases, Buffalo, N. Y.)

Published in *Cancer Research*, 1:653-654. 1941; abstract p. 672.

TUESDAY EVENING SESSION—APRIL 15, 1941

DR. CARL VOEGTLIN, PRESIDENT-ELECT, PRESIDING

SYMPOSIUM ON THE MECHANICS OF CANCER [1]

A CONSIDERATION OF FACTORS WHICH PROMOTE AND HINDER THE GROWTH OF CANCER. ALBERT CLAUDE. (Rockefeller Institute for Medical Research, New York, N. Y.)

[1] The papers presented and the discussion of the topics have not been abstracted.—Editor.

SOME FACTORS WHICH INFLUENCE THE GENETIC MECHANISM OF CANCER FORMATION IN MICE. C. C. LITTLE. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

DETOXIFICATION OF CHEMICAL CARCINOGENS. C. P. RHOADS. (Memorial Hospital, New York, N. Y.)

WEDNESDAY AFTERNOON SESSION—APRIL 16

DR. CARL VOEGTLIN, PRESIDENT, PRESIDING

CUTANEOUS NEOPLASTIC RESPONSES ELICITED BY ULTRAVIOLET RAYS IN HAIRLESS RATS AND THEIR HAIRED LITTER MATES. W. C. HUEPER. (Warner Institute for Therapeutic Research, New York, N. Y.)

Published in *Cancer Research*, 1:402-406. 1941; abstract p. 428.

Discussion

H. F. BLUM (Bethesda, Md.): One of the main problems in the attempt to compare the tumors produced in rodents with those in man seems to center around the question of penetration of radiation. Although it is generally believed that the pigmentation which follows sunburn in man serves as a protection against ultraviolet radiation, it has been shown that at least one of the most important factors in this protection is the thickening of the corneum of the skin. This decreases the penetration of the radiation. The most plausible explanation of the difference in reaction of these mice is the filtering action of the thickened corneum which is laid down in the hairless mice.

STUDIES OF TUMORS OF THE SKIN OF MICE PRODUCED BY ULTRAVIOLET RADIATION. H. F. BLUM (by invitation), J. S. KIRBY-SMITH (by invitation), and H. G. GRADY. (National Cancer Institute, Bethesda, Md.)

A method for irradiation of mice with ultraviolet radiation is described which permits continuous control of dosage and intensity, making possible quantitative measurements in absolute units. The method utilizes an intermediate pressure mercury arc in which the relative intensity of the various lines is constant under the conditions of operation. Continuous measurement of radiation is made with a titanium photocell of the integrating type. The photocell is sensitive only to wave lengths shorter than 3150 A. which includes those effective in producing tumors. Data on the incidence of tumors of all types occurring on the ears of strain A albino mice as a result of such irradiation were presented. The conditions of irradiation include independent variation of intensity and time of dosage. The value of the method as a standard technic for the study of tumor production by ultraviolet radiation was discussed.

Discussion

E. V. COWDRY (St. Louis, Mo.): Does the hyperplasia of the epidermis of mice exposed to ultraviolet light increase the thickness of the epidermis to that of the human epidermis? What do we really know about the penetrability of the epidermis to ultraviolet light?

H. F. BLUM: I would like to be able to answer the second question more accurately. We have several measurements of the transmission of ultraviolet radiation by human skin but they are all subject to criticism. There is, however, a general agreement between them.

It is difficult to make physical measurements of the penetration of the skin by ultraviolet light because of the scattering of the light by multiple reflection. The wave lengths with which we are dealing do not penetrate more than 0.1 mm. of human skin—probably less than that. It is possible that up to 30 per cent of the longer wave lengths which are effective in producing sunburn will pass through the epidermis of the untanned skin.

As the skin is irradiated, the corneum thickens two or three times. That cuts down the penetration considerably. In the mice used in our experiments the germinativum of the epidermis thickened until it looked like human epidermis. Penetration by ultraviolet light to the dermis was thereby reduced to some extent after the first irradiation. Up to the present we have no measurements of the penetration of the skin of the mouse by ultraviolet radiation, but are planning to make such measurements.

E. V. COWDRY: Are you not suggesting that perhaps some other ray than the ultraviolet is effective?

H. F. BLUM: I believe that is ruled out completely. Experiments of Rusch, Kline, and Baumann, and of Roffo eliminate the possibility that any radiation of wave length longer than 3,200 A. is a primary factor. There might be some adjuvant effect of radiations of longer wave length though there is no good evidence for this, but I think we can be sure that these longer wave lengths are not involved in the primary effect.

W. CRAMER (St. Louis, Mo.): In Dr. Grady's experiments did all the tumors appear on the ears?

H. G. GRADY: The large majority of tumors, over 95 per cent, appeared on the external ears. A few tumors appeared on the cornea, a few on the tail, and one on the forepaws. It is to be emphasized that in this study animals were sacrificed as soon as we believed tumors were present. The tumors were not allowed to grow to an appreciable size. This may be one reason for the preponderance of sarcoma. Carcinomatous growths usually appeared later or in combination with sarcoma. Killing the animals when the first tumor was detected may also be the reason why we have not observed more tumors in other locations.

Did Dr. Hueper observe any differences in the time of appearance of the sarcomas as compared with the carcinomas?

W. C. HUEPER (New York, N. Y.): Epidermal responses were usually the first ones we observed. The sarcomatous responses came later, in general. Perhaps there was a difference of a month. Sarcomas involved mainly the orbit, the top of the nose, and the forepaw.

EFFECT OF CLIMATIC ENVIRONMENT UPON THE GENESIS OF SKIN CANCERS INDUCED WITH METHYLCHOLANTHRENE AND UPON THE

GROWTH OF A TRANSLPANTABLE SARCOMA IN C3H MICE. E. W. WALLACE and C. A. MILLS. (University of Cincinnati School of Medicine, Cincinnati, Ohio.)

Skin cancers have been shown to be of more frequent occurrence in the South than in the North. Their development in experimental animals has also been shown to be stimulated by exposure to various types of light irradiation, leading to the somewhat general belief that it is irritation from certain portions of the more intense sunlight in southern regions that is responsible for the higher skin cancer rate there prevailing. We decided, therefore, to study skin sensitivity to methylcholanthrene under artificial conditions that mimic tropical and temperate climates in respect to temperature and humidity but with all effects of light or other forms of irradiation excluded from the picture. The findings we present show clearly that animals kept in depressing moist warmth (92° F., 70 per cent R. H.) are decidedly more prone to earlier development of methylcholanthrene cancers of the skin than are animals kept at 65° F. Subcutaneous inoculation of a highly malignant sarcoma likewise grew rapidly in the skin of animals kept in tropical heat, while those in the cold environment grew more slowly and in over 50 to 80 per cent of cases actually disappeared. Intramuscular injections of the sarcoma, on the other hand, result in tumor rates of development and growth which are approximately equal under the two artificial climatic conditions. It would thus seem that the greater tendency to skin cancers in tropical climates is not based upon skin irritation by the more intense sunrays, but more likely depends upon the richer skin blood supply (and consequent more active skin metabolism) necessitated by the greater difficulty of the body to dissipate heat in the hot climate.

Discussion

E. T. BELL (Minneapolis, Minn.): I would like to call attention to a somewhat similar phenomenon which is observed when mice are injected intraperitoneally with bacteria, such as pneumococci. They live a much shorter time in a hot than in a cold room.

H. L. STEWART (Bethesda, Md.): It has occurred to me that Dr. Wallace's results would have been more striking if a smaller amount of the carcinogenic hydrocarbon had been used. A dosage of 2 mgm. is rather large.

E. W. WALLACE: We are aware of the fact that the dose used was high. We tried using a smaller dose, 0.1 mgm., but the tumors produced were not as numerous. We plan to use smaller doses in future experiments. However, the fact that differences occurred with the large dose used is in itself interesting.

C. A. MILLS: What temperature was used in demonstrating that mice inoculated with pneumococci lived a shorter time in a hot room than in a cold room? Were the animals put directly into the hot room?

E. T. BELL: About 97° F. The animals were put directly into the hot room.

C. A. MILLS: Of course, at 97° F the animal will develop a severe fever. We used temperatures low enough to avoid the production of fever in the mice. Under such conditions, if we put mice newly into these rooms, there is no difference in their death-times after injections of virulent pneumococci. If we adapt them, as we did these animals, during a period of three weeks before injecting the pneumococci intraperitoneally, then they show a markedly greater ability to resist infection at 65° F. than at 91° F.

ADENOCARCINOMA OF THE SMALL INTESTINE AND OTHER LESIONS IN MICE OF DIFFERENT STRAINS RECEIVING ORAL ADMINISTRATION OF 20-METHYLCHOLANTHRENE AND 1,2,5,6-DIBENZANTHRACENE. EGON LORENZ and HAROLD L. STEWART. (National Cancer Institute, Bethesda, Md.)

In experiments, reported before this Association last year, adenocarcinoma of the small intestine was induced in strain A backcross and strain A mice by oral administration of aqueous olive oil emulsions containing 20-methylcholanthrene and 1,2,5,6-dibenzanthracene respectively. Continuation of these experiments revealed that adenocarcinoma of the small intestine can be induced in other strains of mice, namely, C57 brown, C57 black, C3H, and Dba, by administering a stabilized olive oil emulsion orally. Data were presented showing the daily consumption of emulsion and the minimal amount necessary for tumor induction. Other lesions observed in these experiments including sarcoma of the mesentery were described.

Discussion

W. C. MacCARTY, SR. (Rochester, Minn.): Were there any tumors of the large intestine in this series of animals?

E. LORENZ: There were a few animals with tumors in the large intestine. We have observed tumors in the upper part of the colon in a few cases and in the cecum in one case. At present we do not wish to make a statement as to whether these were induced or were spontaneous tumors.

J. G. KIDD (New York, N. Y.): Did the tumors arise as discrete nodules or on the basis of a general tissue disturbance?

H. L. STEWART: We observed two types of tumors of the small intestine as a result of this treatment. One was a localized lesion, a sharply circumscribed, round area with a depressed center. This progressed to form an annular or semi-annular tumor extending into the mesentery and occasionally metastasizing to the base of the mesentery or to the mesenteric lymph nodes. The other type was more diffuse. In some mice we observed a segment of intestine measuring from 5 to 8 cm. in length in which the intestine had the appearance of a garden hose, resembling that seen in lymphosarcoma in human beings. In this diffuse type there were multiple glandular tumors, and multiple early lesions in which a neoplastic change was evolving.

L. C. STRONG: If the tumor of the cecum occurred in a strain A mouse it might have been spontaneous, because cecal tumors occur spontaneously in mice of this strain.

THE PRODUCTION OF TUMORS BY TOBACCO TARS. CURTIS M. FLORY. (University of Chicago, Chicago, Ill.)

Published in *Cancer Research,* **1**:262-276. 1941; abstract, p. 329.

Discussion

H. A. DAVENPORT (Chicago, Ill.): Was the experiment controlled by means of distillates of other plants, such as alfalfa?

C. M. FLORY: Although experiments with distillates of alfalfa were not completed it may be that tumors similar to those produced by distillates of tobacco might be obtained with alfalfa tars. It is possible that substances capable of producing papillomas may be obtained by fractional distillation of practically any organic material of this nature.

E. LORENZ (Bethesda, Md.): At the National Cancer Institute we have made a number of measurements of the burning temperatures of tobacco in pipes, cigars, and cigarettes. Wire thermocouples were used and corrections were made for conduction of heat by the wires. During smoking the temperature in a briar pipe may rise to 800° C. The temperature of a burning cigar goes above 900° C, and in cigarettes rises above 1,000° C.

DR. W. C. MacCARTY (Rochester, Minn.): Have you made any attempt to compare the dosage of tobacco tar necessary to produce papilloma of the ears of the rabbit with the amount of similar material taken in by the ordinary moderate smoker?

C. M. FLORY: We made no attempt to do that.

S. ROTHMAN (Chicago, Ill.): It would seem advisable to conduct experiments of this sort upon animals having certain infectious diseases or other disorders. Every clinician is familiar with the fact that the habit of heavy smoking in the presence of a syphilitic infection predisposes to leukoplakia and consecutive cancer of the mouth and tongue. Would similar experiments with tobacco tar carried out upon syphilitic rabbits give a larger number of malignant tumors?

C. M. FLORY: Such experiments would be interesting, but we did no work along such lines.

E. LORENZ: Dr. Winterstein in Basel obtained results similar to those of Dr. Flory. However, when he painted the ears of rabbits with coal tar first, in an amount insufficient to produce papilloma, and followed this with the application of tobacco tar, carcinomas or sarcomas were produced. He claims that although tobacco tar may be noncarcinogenic in itself, it may become active in combination with other carcinogenic agents.

C. M. FLORY: That point should be further investigated.

H. G. GRADY (Bethesda, Md.): Do the lesions referred to as "carcinomatoid" regress after the painting with tobacco tar is stopped?

C. M. FLORY: I am not able to answer that question because in these experiments no attempt was made to observe the final condition of these lesions. Most of these tumors were found when the animals died. Rous and Kidd have reported that carcinomatoid tumors regress. The absence of metastases was one of the chief reasons for our calling these tumors "carcinomatoid."

E. V. COWDRY (St. Louis, Mo.): Does tobacco tar have a greater carcinogenic action when applied to the mucous membranes than when it is applied to the skin?

C. M. FLORY: I have no information on that point.

J. G. KIDD (New York, N. Y.): What were the early changes produced in the skin of rabbits painted with tobacco tar?

C. M. FLORY: Marked hyperkeratosis, peeling of the skin, and loss of hair were produced as early as 2 weeks after the start of painting. We peeled off this hyperkeratotic skin to obtain a fresh surface for painting.

THE PRODUCTION OF TUMORS IN EXPERIMENTAL ANIMALS WITH SULFANILAMIDE. ALF T. HAEREM. (University of Chicago, Chicago, Ill.)

Spindle cell sarcomas developed at the site of injection in 2 out of 20 Swiss Albino mice (Bar Harbor) injected with 15 mgm. of sulfanilamide in 0.5 cc. of lard. The animals with tumor were killed at 222 and 262 days. Of 10 stock albino rats given injections of 15 mgm. of sulfanilamide in lard on 2 occasions, 2 developed sarcomas. These tumors occurred at 8 months and 13 months. Controls included 10 rats similarly injected with aspirin in lard, 10 receiving no injections, and 30 injected with lard alone. No tumors occurred in these groups.

Tumors were not produced by feeding rats a stock diet containing 2.5 per cent sulfanilamide, during a test period of 16 months. These animals, however, failed to gain weight, because of a smaller dietary intake, and they failed to produce litters.

A preliminary report has been published in *Proc. Soc. Exper. Biol. & Med.,* **45**:536-539. 1940, and a more extensive report will be made later.

In the absence of Dr. Haerem, this paper was presented by Dr. Paul E. Steiner.

Discussion

C. VOEGTLIN (Bethesda, Md.): I think it is important for this Association to see to it that these findings are not misinterpreted by the press and by the laity. Naturally, it would be wrong to say that sulfanilamide and other compounds of this series actually produce cancer in human beings. It might create an unfortunate situation.

It is obvious that these findings will have to be followed up further. In fact there is even more extensive work to be done because there are numerous chemicals being discovered all the time, some of which, in animals at least, might produce cancer.

G. O. GEY (Baltimore, Md.): Since June, 1938, we have been using sulfanilamide and other sulfonamide drugs in tissue cultures as a protection against and for the treatment of infection. We have used concentrations of sulfanilamide from 5 mgm. to 800 mgm. per cent; *i.e.,* up to the point of full saturation. In only a few cultures after long-continued exposure of cells to sulfanilamide have we produced any changes. One of the things that we observed is a disappearance of granules with the production of very

clear cells. Sometimes there is hypertrophy of cells. Such altered cultures, when inoculated into animals, have not produced tumors.

P. E. STEINER (Chicago, Ill.): When Dr. Haerem showed me these results a little over a year ago, I thought that they were so important it would be advisable immediately to begin to make a check on them by using a different strain of mice, a different brand of sulfanilamide, different diet, and other different conditions. The experiments begun 9 months ago are now reaching a critical stage. So far there have been no tumors.

O. M. GRUZHIT (Detroit, Mich.): Usually the injected sulfanilamide is absorbed within 24 hours. How is the carcinogenic mechanism accounted for in view of the fact that the material is not retained at the site of injection?

P. E. STEINER: It was incorporated in lard deliberately to hold it at the site of injection.

M. E. KRAHL (Indianapolis, Ind.): Have you tried any of the more insoluble derivatives of sulfanilamide, such as sulfapyridine and sulfathiazole?

P. E. STEINER: Dr. Haerem had a great many of these experiments going on. They were partly interrupted by his having been called to military service. I have no definite information on their present status.

J. E. EDWARDS (Bethesda, Md.): Were there any hepatic changes in the animals administered sulfanilamide?

P. E. STEINER: We have seen hepatic changes but do know how to interpret them. As hepatic changes in these animals are fairly common, we doubt if the changes noted are significant. Dr. Haerem has also noted interesting renal changes.

M. J. SHEAR (Bethesda, Md.): With reference to Dr. Edwards' question, since 1934 we have been testing a large number of compounds of this type; *i.e.*, nitrogen-containing benzene derivatives. Dr. Stewart has examined the histological sections. There have been lesions of the liver, and in some cases tumors have been produced by compounds of this type.

The interesting findings just presented bring to the fore a question that has been under discussion for a number of years. When the Scottish workers obtained sarcomas on the injection of a trypanocidal drug (styryl 430), and when the butter yellow dyes, which are still used in some places to color foods, were found to be carcinogenic, and when some of these simpler benzene derivatives related more or less closely to the great variety of dyes, drugs, and food preservatives in common use were found to produce changes of this type in long-term experiments, the question came up as to whether a systematic survey of such substances should be undertaken. The matter was discussed with Dr. Calvary of the Food and Drug Administration. It had been hoped at that time that it might be possible to arrange for such a survey. This was not possible because of the magnitude of the task. This would not be a new research approach but a routine, long-term testing of many substances for carcinogenic potency.

Since this work would be too large an undertaking for a single laboratory the question came up as to whether some central committee might perhaps be set up by this Association to serve as a central clearing agency for information and to assist in securing cooperation among laboratories interested in testing these compounds. The Committee could help to avoid multiplication of tests of the same compound in different laboratories and aid in making the survey so broad that eventually we might have information on the substances in common use.

This same matter was brought up by Dr. Steiner a few months ago. I wonder what the President would suggest in regard to the appointment of such a committee.

C. VOEGTLIN: Are you in favor of a motion that this question be referred to the Board of Directors?

M. J. SHEAR: I so move.

W. C. MacCARTY, SR. (Rochester, Minn.): I second it.

The motion was put to a vote and carried.

EXPERIMENTAL LIVER CANCER AND ITS INHIBITION BY VARIOUS FOOD SUBSTANCES. K. SUGIURA and C. J. KENSLER. (Memorial Hospital, New York, N. Y.)

In the present study animals were fed 20 cc. of a 3 per cent solution of butter yellow (dimethylaminoazobenzene) in olive oil mixed with 1 kg. of unpolished rice, supplemented with carrot.

As a preliminary study, the susceptibility of rats of different strains to the carcinogenic action of butter yellow was determined. The susceptibility of rats of different strains to the carcinogenic action of butter yellow is not the same. In the case of the Sherman strain, 10 per cent of the rats showed liver cancer at the end of 120 days on butter yellow rice diet. In the case of the Wistar strain, 100 per cent of the rats showed liver cancer at the end of 150 days, and in the case of the Evans strain, 100 per cent of the rats showed liver cancer at the end of 220 days.

The liver cancers were found to be various histological types of hepatomas, namely, glandular or cholangioma type, trabecular or solid type, and mixed type. Cirrhosis with adenomatous hyperplasia of bile diets preceded these malignant tumors.

At the end of 1 month of butter yellow feeding, the livers appeared normal. At the end of 2 months of butter yellow feeding, the livers showed a marked uneven surface with more or less well-defined nodules. At the end of 3 months of butter yellow feeding, the livers showed many large well-defined nodules. At the end of 5 months of butter yellow feeding, the livers showed large tumor nodules.

After one month of butter yellow feeding, section of a liver shows swelling of cells, much narrowing of sinusoids, and smooth surface of the capsule. After two months' feeding, section shows annular cirrhosis or adenomatous hyperplasia of bile ducts, beginning cholangioma. After three months' feeding, section shows cholangioma or hepatoma or both.

The effect of various food substances on the production of liver cancer by butter yellow was investigated.

Dietary supplements of casein, corn oil, cod liver oil, wheat germ oil, lecithin, and choline had no inhibiting effect upon the production of liver cancer.

Butter yellow did not produce liver cancer in rats fed on a rice diet containing 15 per cent of brewer's yeast.

The inhibiting effect of yeast feeding upon the production of liver cancer is distinctly diminished when the basal diet of butter yellow rice contained less than 15 per cent yeast. Thus with the addition of 6 per cent yeast to the butter yellow rice diet, 30 per cent showed liver cancer. With the addition of 3 per cent yeast to the butter yellow rice diet, 70 per cent showed liver cancer.

It is interesting to note that 3 per cent of yeast in a synthetic diet deficient only in vitamin B complex was sufficient to promote normal growth in rats. But that amount of yeast is not sufficient to prevent the production of liver cancer by butter yellow. Similarly, it was found that the addition of 5 per cent liver extract to the butter yellow rice diet had a complete inhibiting effect, while the addition of 1 per cent liver extract inhibited only 35 per cent.

Livers of rats fed with 15 per cent yeast show normal appearance at the end of 300 days, while livers of rats on butter yellow rice diet without the supplementary yeast show many cancer nodules in all animals at the end of 150 days.

As both yeast and liver are rich in vitamins of the B group and as it was found that our basal unpolished rice diet contained only minimal quantities of riboflavin, several experiments were undertaken in which riboflavin was fed. The administration of large amounts of riboflavin alone is without significant protective effect. However, when a relatively small amount of riboflavin (200 µgm.) was fed with casein, almost complete protection was afforded the animals at 150 days.

MORPHOLOGY OF HEPATIC TUMORS IN RATS FED P-DIMETHYLAMINOAZOBENZENE (BUTTER YELLOW). JESSE E. EDWARDS and JULIUS WHITE. (National Cancer Institute, Bethesda, Md.)

Over two hundred Osborne-Mendel male and female rats were fed p-dimethylaminoazobenzene for various periods of time with the production of tumors in the majority of animals.

The tumors arising in the liver are usually multiple, white, pearly gray, pink, or purple, with solid and cystic areas. Frequently one of the tumors is appreciably larger than the others. Peritoneal implants occur with extension of these secondary foci into other organs such as the spleen, pancreas, and diaphragm. Metastases to lungs, retroperitoneal and mediastinal lymph nodes are found.

The tumors may be successfully transplanted subcutaneously and intraperitoneally into rats of the Osborne-Mendel strain.

The tumor tissue may be divided into three histologic types but frequently mixtures of these are present in the same tumor.

Type I.—The tumor cells are morphologically not unlike regenerating hepatic cells and occur in cords along endothelial-lined sinusoids. Mitotic figures are uncommon and evidence of nuclear abnormality is frequently lacking. The regularity of this picture makes it difficult to distinguish this type of tumor from hepatic regeneration. A distinguishing feature of this type of growth is invasion of blood vessels. Metastases and peritoneal implants from this type of tumor have not been encountered, nor have we successfully transplanted this type.

Type II.—This is the most common type. The cells vary somewhat in appearance from those having no distinguishing features to those with sharp cell margins somewhat suggestive of hepatic cells. The cytoplasm is faintly basophilic and the nuclei are relatively small, each showing a prominent nucleolus. The relation of the tumor cell collections to blood vessels is characteristic. Each aggregation of tumor cells is surrounded by a delicate basement membrane which separates it from the endothelium of adjacent blood sinusoids.

The relation of the tumor cells to each other varies so that sheets of cells, cords, or acini are found.

Type III.—This type grows as an adenocarcinoma which is not specific either in the appearance of the cells or in their relation to the connective tissue and blood vessels. Some of the cells contain droplets of mucin and some acini may be distended with this substance to present the picture of a colloid carcinoma. Papillary types of adenocarcinoma are also encountered. Type III tumor is practically always mixed with tumor of the second type.

By some investigators this adenocarcinoma type has been called cholangioma, implying or indicating that it is derived from the epithelium of bile ducts. That induced adenocarcinoma of the rat's liver may in reality be of hepatic cell origin has several points of support, as follows:

1. Among the Japanese workers, Kinosita, studying early lesions, feels that regardless of whether a tumor develops into a typical hepatic cell carcinoma or a so-called cholangioma, it is derived from hepatic parenchyma and not from bile ducts.

2. The almost constant association of adenocarcinoma with tumor generally conceded to be of hepatic cell origin and the presence of transition forms between these two types of tumor would suggest that adenocarcinoma may merely be a variant of tumor derived from hepatic cells.

3. The study of one case in several generations of transplantation has convinced us that this may be so. In the primary form the neoplastic tissue was growing as types I, II, and III. In the first two generations of transplantation it was growing as adenocarcinoma. In the third generation it was growing partly as adenocarcinoma and partly as a solid tumor consistent with one of hepatic cell origin. There were transitional forms. In the fourth and fifth generations the tumors were completely solid and entirely consistent with a tumor of hepatic cell origin.

4. The hepatic parenchyma is developed from an outpocketing of the gut which would make it conceivable for hepatic parenchymal cells to form glands when growing as neoplastic tissue.

Discussion

H. L. STEWART (Bethesda, Md.): I know of no field in experimental cancer research in which the opinion of the well trained, experienced pathologist is of more importance than in evaluating the presence or absence of a tumor of the liver in an animal fed one of the azo dyes. My own experience has indicated that there is a large group of cases in which it is questionable whether or not

a tumor existed. On the other hand, nearly all investigators in reporting their results show tables of figures on the presence or absence of tumor but in which there is usually no mention of a doubt whether a given lesion is neoplastic or nonneoplastic.

Regarding the possible origin of these tumors from bile ducts another reason against such a supposition is that if the azo dyes are carcinogenic for the epithelium of the intrahepatic bile ducts they should also produce tumors of the larger extrahepatic bile ducts, or even of the gall bladder in the mouse. This has not been observed.

W. C. MacCARTY, SR. (Rochester, Minn.): There are many artifacts in the histological sections which have been shown in the slides illustrating these papers. These could be avoided by better technic, following methods I described some 37 years ago and have been using successfully ever since. I have rarely seen these methods used outside my own institution. One important part of the technic is to use perfectly fresh tissues.

Following these remarks there was a general discussion of histological technic.

E. V. COWDRY (St. Louis, Mo.): It seems to me that in the differentiation of tumors possibly arising from bile ducts as contrasted with tumors possibly arising from parenchymatous hepatic cells, it would be useful to make preparations to demonstrate bile capillaries. Bile capillaries are, of course, formed between the hepatic cells. They would not, I think, be formed by bile duct cells. It seems to me that even an abortive formation of bile capillaries might be significant.

W. H. WOGLOM (New York, N. Y.): When I began looking at these tumors I thought I could distinguish the bile duct type from the parenchymal type, but with more experience, I became doubtful about the possibility of making this distinction. I have seen a tubule with one half resembling bile duct epithelium and the other half resembling parenchyma.

J. WHITE: In connection with the work of Drs. Sugiura and Kensler, I believe that most investigators have not taken into account sufficiently the nutritional requirements of the tumor and the relationship of the nutritional state of the animal as a whole to the occurrence and growth of tumors. Some years ago Dr. Voegtlin and his collaborators showed that by removing some of the essential amino acids from the diet the growth of certain tumors was retarded. At the same time the growth of the animal itself was retarded.

In regard to the statement in this paper that supplementing the diet with riboflavin and casein will prevent the formation of liver cancer, I wonder what would have happened if the animals had been allowed to ingest this diet over a period longer than 150 days. Is the result reported merely a retardation of the latent period of tumor formation?

Using a somewhat different diet in similar experiments we have found that if we allowed the animals to ingest butter yellow, tumors would arise in about 4½ to 6 months. On the other hand, if we removed one of the dietary essentials (cystine), under the conditions of our experiment, no tumors were formed before 7½ months. Usually the tumors which appear at that time are very small. I feel that this result is due to the fact that the animal's resistance is lowered by a very poor diet at the start.

The animals will usually have the same weight at the end as at the start of the experiment. As a result two conflicting processes are taking place. One is the tendency to detoxify the toxic agent (butter yellow in this case) and the other is the tendency to form tumors. Under conditions of poor nutrition it is impossible for the first and natural process of detoxification of the agent to take place completely. As a result, enough of the carcinogenic agent is still present to produce these tumors. The addition of a material such as casein probably supplies the sulfur-containing amino acid needed to detoxify the butter yellow present in the liver. I venture to say that tumors would be formed if these animals were continued on the experimental diet for periods longer than 150 days.

K. SUGIURA: In a previous communication, it was shown that the development of liver cancer in rats following oral administration of p-dimethylaminoazobenzene is completely inhibited by daily ingestion of about 1.2 gm. of brewer's yeast. All of the 54 rats examined between 150 and 300 days after the beginning of the experiment showed smooth and practically normal livers. In contrast, the animals fed the butter yellow rice diet without yeast developed typical liver cancer in all cases in a comparatively shorter period of time (100 to 170 days). Some of the experiments with butter yellow rice diet containing 15 per cent of yeast were continued for 450 days. In that time there was complete inhibition of tumor formation in 75 per cent of the animals, while 25 per cent showed tumors. In the experiments with riboflavin and casein, 95 per cent of the animals were protected for 150 days. Perhaps if we had kept the animals longer more would have developed tumors, but probably not 100 per cent. We are making experiments to test the inhibiting action of combinations of riboflavin with cystine or methionine.

J. LOCKWOOD (Philadelphia, Penn.): It would be of considerable importance to know something about the chemical composition of these livers during the weeks preceding the development of the tumors. Ravdin and his associates in Philadelphia, and Whipple in Rochester, have shown that in rats with livers having a high content of fat the administration of a hepatotoxic agent such as chloroform or arsphenamine in acute experiments will produce extensive necrosis, and that this type of liver injury can be prevented by adjusting the diet in such a way as to maintain a high protein content in the liver. Furthermore, Ravdin has shown also that the production of sterile subcutaneous abscesses in animals will protect the liver against injury by hepatotoxic agents. It is possible that these phenomena may have a bearing upon the production of tumors of the liver by butter yellow, where the injury is chronic rather than acute.

M. J. EISEN (New York, N. Y.): Did liver cancer occur in the absence of cirrhosis?

K. SUGIURA: o-Aminoazotoluene and dimethylaminoazobenzene are equally effective when given orally in producing liver cancer in rats. This change occurs after

300 days when o-aminoazotoluene is used and after only 150 days with dimethylaminoazobenzene. The sequence of events is not the same with both dyes. In the case of dimethylaminoazobenzene, cirrhosis almost always precedes cancer of the liver. With o-aminoazotoluene the stage of cirrhosis is less prominent. When yeast is fed cancers are often not preceded by cirrhosis.

M. J. EISEN: May it not be that the yeast, the vitamin complex, prevents the cirrhosis of the liver, which is apparently a determinant of tumor production in a large majority of the rats? Possibly the bile ducts rarely if ever become cancerous because butter yellow is not in itself directly carcinogenic. It does not produce cancer at the site of injection. Butter yellow may produce liver damage, which is followed by cirrhosis, before the cancer occurs. In human beings primary cancer of the liver is more common in cirrhotic livers than in normal livers. The vitamin B complex prevents the cirrhosis of the liver, but does not function directly as an inhibitor of carcinogenesis.

M. J. SHEAR: As Dr. Eisen has stated, tumors have rarely been reported at the site of injection of these azo dyes. They are absorbed into the circulation and produce tumors of the liver. On the other hand, methylcholanthrene and benzpyrene which are actively carcinogenic when applied to the skin or subcutaneous tissues are almost completely inactive when applied within the liver of the rat. So far in experiments extending over $1\frac{1}{2}$ years no tumors have been produced by these hydrocarbons injected or implanted into the liver.

M. J. EISEN: The main reason for the nonproduction of organ tumors is the encapsulation of the hydrocarbon. In some experiments the connective tissue encapsulating the hydrocarbon in organs was changed to spindle cell sarcoma.

M. J. SHEAR: Have you gotten spindle cell sarcoma in the rat?

M. J. EISEN: Not as yet, but with the proper technic the carcinogens should be capable of eliciting various forms of organ tumors.

M. J. SHEAR: The peculiar feature we have noted is that the connective tissue around the hydrocarbon in the liver does not go into malignancy whereas it does at other sites.

BEHAVIOR OF PARAMECIUM MULTIMICRONUCLEATUM AFTER LONG ADAPTATION TO METHYLCHOLANTHRENE.

R. R. SPENCER and M. B. MELROY. (National Cancer Institute, Bethesda, Md.)

For more than 18 months a strain of *Paramecium* has been exposed continuously to methylcholanthrene (10 mgm. per 1 cc. of medium). During this period, more than 50 transfers of the cultures have been made at approximately 10-day intervals covering at least 300 successive cell divisions. When removed from the methylcholanthrene, washed, and placed under conditions of partial starvation, the adapted organisms have shown an increased ability to survive and have multiplied to a far higher population level than untreated organisms. These changes have held for 10 transfers away from methylcholanthrene, suggesting some degree of permanency. Neither of these functional changes appeared in the first few transfers of the adaptation process and the ability to survive appeared earlier than did the high population levels. This delayed response is considered significant. No claim is made that the methylcholanthrene-adapted *Paramecia* are analogues of mammalian cancer cells induced by the same agent; but it is believed that a study of small organisms exposed for long periods through many cell cycles to carcinogenic agents may throw light on the mechanism of carcinogenesis in mammalian tissues.

Discussion

E. V. COWDRY (St. Louis, Mo.): I understood from the graphs shown that there is no evidence of inhibition of growth or other toxic action by methylcholanthrene on the *Paramecia*. Do *Paramecia* divide more frequently and rapidly under the influence of methylcholanthrene?

R. R. SPENCER: We have no evidence whatever that methylcholanthrene in the amounts used (1:1,000,000) has an inhibitory action upon the growth of *Paramecium*. Previously we and others, using *Paramecium* and bacteria, have found that the initial effect of methylcholanthrene was a stimulation of cell division. In these curves, however, you will note that this response (high population levels, not to be confused with the rate of cell division) was delayed, came after many transfers, and did not reach its peak until about the 12th day.

We have not made observations on individual organisms to determine whether cell division was faster than in the normal rate.

M. E. KRAHL (Indianapolis, Ind.): Were the cultures of *Paramecium* in solutions of the hydrocarbon kept in the dark or were they exposed to daylight?

R. R. SPENCER: In the laboratory where these cultures are studied, electric lights are covered with five layers of yellow cellophane which excludes the ultraviolet radiations. All the windows are blacked out. The experiments, however, were started in daylight. The cellophane covers were put on the lights and the windows darkened at about the time of the 25th transfer as an extra precaution. We had not noticed any evidence of photodynamic sensitization in the diffuse daylight of the laboratory.

CANCER OF THE MOUTH. I. SOME CHEMICAL ASPECTS OF BUYO-CHEEK CANCER.

W. C. WOELFEL (by invitation), JOHN W. SPIES, and J. K. CLINE. (University of Texas School of Medicine, Galveston, Texas.)

In the Far East the habit of betel chewing is a factor of major importance as a cause of cancer of the mouth. The present report is an attempt to develop a theory of the origin of betel chewer's cancer, based on a consideration of the chemical constituents of the chew.

The chew consists primarily of a piece of areca nut, wrapped in the leaf of the betel vine, together with some lime. In certain localities other ingredients are added, such as gambir, catechu, tobacco, or cloves. By comparison of descriptions of ingredients it is possible to eliminate from

consideration all except those common to the quid chewed in all localities. The remaining common ingredients are the areca nut, the betel leaf, and lime. Attention is to be focussed on compounds of the nut and leaf, rather than on the lime.

The fat obtained from the nut by extraction with ether did not produce tumors in mice in 15 months. Injection of the unsaponifiable fraction also gave negative results after 10 months. Alcoholic extracts of the nut produced severe ulcerations, probably because of the tannin present. Similar extracts from which the tannin had been removed gave negative results.

There are at least four allyl phenols in the essential oil from the betel leaf. These are: chavibetol, chavicol, allyl pyrocatechol, and methylengenol (allyl veratrole). Allyl compounds of this type are readily isomerized to propenyl compounds, the double bond shifting toward the benzene ring. Such propenyl compounds undoubtedly occur in the leaf. The propenyl compounds are very reactive and tend to dimerize spontaneously. This conversion probably takes place within the leaf under the influence of sunlight and enzymes. An example is the "photoanethole" of DaVarda, formed by the action of sunlight on anethole, obtained from anise oil. This was shown later to be dimethoxystilbene. Other stilbenols have been isolated from the sulfite liquors of wood, and it is thought that similar compounds occur in betel leaf, areca nut, and tobacco. Compounds having high estrogenic activity are formed when anethole is demethylated by alcoholic potassium hydroxide. Dianol, prepared by demethylation of "isoanethole" produced estrus in castrated female rats in doses of 0.1 mgm.

Dimeric compounds similar to these may be present in the betel leaf. The possible compounds and their probable estrogenic properties were discussed.

We have tested a dimer of anethole known as "methanole" for estrogenic activity and found it to be negative in 60 mgm. doses. Its dimethylation product is being investigated. We have found also that diisoeugenol has no estrogenic activity in 15 mgm. doses.

We are engaged in the synthesis of possible dimers of betel leaf phenols, and plan to test them for estrogenic and carcinogenic activity.

Discussion

F. C. TURNER (Bethesda, Md.): I might state that I tested 6 of these allyl compounds for a different purpose; namely, to determine whether they would retard the development of tumors induced by an injection of methylcholanthrene. Mice, which had received a minimal dose of methylcholanthrene in tricaprylin, were injected periodically with these allyl compounds. The results were negative.

CELL DIVISION. WARREN H. LEWIS and MARGARET REED LEWIS. (Wistar Institute of Anatomy and Biology, Philadelphia, Penn.)

Read by title; abstract not available.

THE INHIBITION OF PHOSPHOLIPID OXIDATION BY CARCINOGENIC AND RELATED COMPOUNDS. H. P. RUSCH and B. E. KLINE. (University of Wisconsin, Madison, Wis.)

Published in *Cancer Research,* 1:465-472. 1941; abstract p. 509.

Discussion

M. E. KRAHL (Indianapolis, Ind.): We are very much interested, as is the Wisconsin group, in the reactions of carcinogenic agents with lipoid materials. At the same time we have been studying the solubility of the hydrocarbons in water. While they are commonly considered insoluble this is not really so. They have substantial solubilities.

The important point for enzyme studies is that the hydrocarbons can be carried into solution quite readily in the presence of proteins. This makes it possible for these substances to take part in enzyme reactions in cells, in aqueous solutions, where activity coefficients are greatly increased by proteins. Therefore, we should not give up hope of study of the aqueous systems.

C. VOEGTLIN (Bethesda, Md.): The speaker referred to arsenic as a carcinogenic agent. I believe there is no clear-cut experimental evidence to the effect that arsenic produces tumors. We have some work under way to see whether certain arsenicals can produce tumors. So far the results have been negative.

E. ALLEN (New Haven, Conn.): I was much interested in seeing Drs. Rusch and Kline tie up estrogens and carcinogens in these oxidation reactions. The estrogens are known to affect color in feathers and skin. There is probably some tie-up also with melanotic tumors. This promises to be a very interesting and productive field of investigation.

WEDNESDAY AFTERNOON SESSION—APRIL 16

DR. CARL VOEGTLIN, PRESIDENT, PRESIDING

THE RELATION OF DIET TO BENIGN NEOPLASIA (ULCERO-PAPILLOMAS) OF THE RAT'S STOMACH. A. BRUNSCHWIG and R. A. RASMUSSEN. (University of Chicago School of Medicine, Chicago, Ill.)

Published in *Cancer Research,* 1:371-378. 1941; abstract, p. 429.

Discussion

W. CRAMER (St. Louis, Mo.): The lesions described by Dr. Brunschwig are very similar, as far as the forestomach is concerned, to lesions that I have seen in rats when kept on certain diets. But I came to the conclusion that no specific diet is responsible for their occurrence.

I never observed the lesions in the glandular portion of the stomach. While diet may be a factor responsible for such lesions, the age of the rats may have some influence and a genetic factor also may be involved. When I repeated my own experiments some years later using young rats and keeping them for several months, I did not observe these lesions in the stomach, although the animals

were kept on exactly the same diets on which 3 years earlier the lesions had appeared.

A. BRUNSCHWIG: We have used about 200 rats in these experiments. Differences in ages of the animals may account for some of the discrepancies in experimental results. In mature rats, 1½ to 2 years old, we found it took several weeks before the lesions would appear. After preliminary observation we used young rats weighing 60 to 130 gm. They developed lesions regularly, and as early as 12 days in some cases, when fed the diet of boiled rice only. With rats weighing 200 to 250 gm., 4 to 5 weeks were required for the development of the lesions.

THE PRODUCTION OF TUMORS WITH AN EXTRACT FROM HUMAN LIVER. PAUL E. STEINER. (University of Chicago, Chicago, Ill.)

The nonsaponifiable lipid fraction obtained from the livers of persons having cancer, the livers themselves containing no cancer, produced sarcomas at the site of subcutaneous injection in mice. The extract was obtained by saponification and extraction with ethylene dichloride. It was administered in one dose of 0.5 gm., dissolved or suspended in sesame oil. The first sarcoma killed its bearer in the 7th month and 11 more tumors developed in 37 mice (effective total), a yield of about 30 per cent. The tumors were transplantable in series in 2 out of 3 attempts. One original induced tumor metastasized to the lung.

A similar extract from the livers of noncancerous persons produced one sarcoma during the 12th month (35 mice living), and another in the 13th month.

Experiments are now under way to study the origin, nature, distribution, and etiologic significance of this carcinogen.

A preliminary report was published in *Science,* **92**:431-432. 1940.

Discussion

A member asked the speaker if he has any idea as to the nature of the carcinogen.

P. E. STEINER: I do not. I can say that about two-thirds of the material is cholesterol.

E. W. WALLACE (Cincinnati, Ohio): Would methylcholanthrene added to the livers appear in the extract prepared by Dr. Steiner?

P. E. STEINER: We have not tried that.

M. J. SHEAR (Bethesda, Md.): I should like to ask Dr. Steiner what he thinks of the possible role of sesame oil as a carcinogen. Some investigators have found that sesame oil alone, without additional carcinogens, produced sarcomas in a few mice. However, I understood that none of the mice in Dr. Steiner's control group, not reported in detail here today, developed sarcoma from injection of sesame oil alone. In the repetition of these experiments it will also be necessary to consider the role of the solvent from the point of view of the presence or absence of promoting or retarding factors in it.

This question came up in Boston several years ago and Dr. Shimkin, at the suggestion of Dr. Fieser, tested a number of synthetic solvents, chiefly triglycerides. If one compares the reproducibility of results, as Dr. Shimkin and we have done, one finds that with very low doses of carcinogens dissolved in vehicles of biological origin, which vary widely in composition, one solvent often gave results different from those obtained with the same amount of the carcinogen in another vehicle. It would be of interest, therefore, to know whether the fractions injected by Dr. Steiner in solution in sesame oil would produce tumors when injected in another vehicle of known composition.

P. E. STEINER (Chicago, Ill.): The sesame oil used in these experiments has been tested by itself. The unheated oil has not produced tumors. Sesame oil heated to 350° C., at which point it changed color and fumed, produced one tumor in one mouse among a large number of animals injected with the heated oil.

Following Dr. Shear's recommendation we have been using tricaprylin as a vehicle. Thus far we have not been favorably impressed because our tricaprylin is caustic and causes greater reaction at the site of injection than does sesame oil.

J. B. MURPHY (New York, N. Y.): Did I understand correctly that the technic used in inoculating controls was different from that used in inoculating test animals?

P. E. STEINER: They received two injections.

J. B. MURPHY: Did you have any controls where you used exactly the same technic in the preparation of the normal liver fraction and when this product was administered in the same doses at the same intervals as in your reported tests?

P. E. STEINER: I am not ready to report on that.

H. G. GRADY (Bethesda, Md.): Were the extracted livers from the cancer patients free from metastases?

P. E. STEINER: Yes, they were both grossly and microscopically free from metastases. We have extracted large quantities of tumor tissue by similar methods and have injected the extracts into mice. During periods up to two years such extracts have not produced tumors.

G. PINCUS (Worcester, Mass.): Was there any fatty degeneration in the livers from the cancer patients?

P. E. STEINER: Yes, but the amount of fatty change in the livers which is visible with fat stains under the microscope is not related at all to the yield of nonsaponifiable lipid from those livers. There is no correlation there.

EXTRACTION OF A CARCINOGENIC FRACTION FROM HUMAN URINE. PRELIMINARY REPORT. R. STEELE (by invitation), F. C. KOCH (by invitation), and P. E. STEINER. (University of Chicago, Chicago, Ill.)

Published in *Cancer Research,* **1**:614-617. 1941; abstract p. 670.

Discussion

F. C. KOCH (Chicago, Illinois): I should like to comment on the unexpected finding that the benzene extract of the nonhydrolyzed urine thus far has not produced cancer in these animals. The amount of this fraction injected at each time was the equivalent of about 2 liters of urine.

The same is true of the benzene extract obtained after butyl alcohol extraction and acid hydrolysis of the urine. It is admitted that this procedure may not appear logical from the standpoint of hoping to extract typical steroids,

androgens, or estrogens by the last benzene extraction. Presumably the free steroids were removed in the first benzene extraction and conjugated steroids in the butyl alcohol extraction.

The butyl alcohol extracts injected per mouse were the equivalents of 82 cc. of urine in the cancer series and 100 cc. in the noncancer series. It is barely possible that androgens or estrogens may be more cancerogenic when in the conjugated form. It is also possible that nonsteroid cancerogens may be present in this fraction.

We did not make any attempt to isolate pure substances from the various fractions because we were making a rough survey for cancerogenic material.

THE *d*-PEPTIDASE ACTIVITY OF SERUM AS A DIAGNOSTIC TEST FOR CANCER. MARY E. MAVER, J. M. JOHNSON, and J. W. THOMPSON. (National Cancer Institute, Bethesda, Md.)

In 1939, Waldschmidt-Leitz and Mayer published a clinical test for cancer based on experiments which showed that the serum of patients with carcinoma always hydrolyzed over 50 per cent of the racemic peptides dl-leucylglycine, dl-glutamylglycine, and dl-glutamylglycine ethyl ester, indicating the ability to hydrolyze the optically unnatural isomer of the racemic peptides. Our experiments with human serums show that *d*-peptidase activity cannot be used as a test for carcinoma since the serum of only 1 of 19 patients with carcinoma reacted positively.

With rat serums it was shown that concentration and activation factors can influence the *d*-leucylpeptidase activity of the serums of normal rats and of rats bearing Jensen.

The *d*-leucylpeptidase activity of the serums of rats was not related to possible *d*-amino acids in the neoplastic tissues since these tissues did not possess *d*-peptidase activity.

Published in the *Journal of the National Cancer Institute*, 1:835-843. 1941.

OBSERVATIONS ON THE OXIDATIVE BEHAVIOR OF TUMORS, ARTIFICIALLY BENIGN TUMORS, AND THE HOMOLOGOUS NORMAL TISSUES. W. T. SALTER, F. N. CRAIG, and A. M. BASSETT, M.D. (Thorndike Memorial Laboratory, Boston City Hospital, and Harvard Medical School, Boston, Mass.)

By the use of succinate and paraphenylenediamine, it was possible to test the activity of the cytochrome system in homologous normal and malignant tissues from the same host. Connective tissue, liver, and muscle were studied, and in all three the malignant form showed low cytochrome-system response. On the contrary, "artificially benign" sarcoma 180 in immunized hosts behaved as if malignant. A few instances were described in which the procedure has been applied to mammary tissue (human and murine); to human leukocytes in health and in leukemia; to human lymph nodes; to tumorous nodules of the human skin; and to Shope virus papilloma. The results suggest that under favorable circumstances this biochemical method of studying animal and human tissue might be a useful adjunct to routine morphological study.

To be published in *Cancer Research*.

Discussion

DEAN BURK (New York, N. Y.): Would you be inclined to regard these results as indicating that malignant tumors are low in cytochrome oxidase and that this is an added characteristic of metabolism of malignant tissue?

W. T. SALTER: Yes, I think this answers the question asked by Dr. Rosenthal yesterday concerning studies of succinate oxidase.

There is said to be a New Haven tumor No. 1 which is high in cytochrome. I hope to study this tumor.

D. BURK: I have been informed by Dr. Potter that quantitative analysis has shown that the New Haven tumor No. 1 is in fact low in cytochrome, and that earlier reports to the contrary had not been based on a quantitative analysis.

E. V. COWDRY (St. Louis, Mo.): Is there any considerable difference in the water content of these types of tissues?

W. T. SALTER: There may be a difference, but this variation is not over 20 per cent.

O. ROSENTHAL (Philadelphia, Penn.): I think that Dr. Salter's work gives further evidence that normal tissue and tumor tissue may have the same type of metabolism. In my investigations I found that the metabolism of normal cartilage closely resembled that of malignant tumors in respect to respiration and succinate oxidase. What was the concentration of succinic acid used in these experiments?

W. T. SALTER: I should have said of course that a good deal of this work is based on the work of Dr. Rosenthal on homogenized tissue. As regards the concentration of substances used, we compromised on 0.2 per cent of succinic acid and 0.2 per cent paraphenylenediamine.

FURTHER OBSERVATIONS ON THE INDUCTION OF SARCOMA FOLLOWING THE INJECTION OF WHEAT GERM OIL. PAUL N. HARRIS. (Indianapolis, Ind.)

Attempts to confirm the observations of Rowntree and associates on the development of sarcoma in rats following oral administration of ether extracted wheat germ oil have been unsuccessful, although administration of large doses daily was carried out over periods of 5 months or more. As a result of intraperitoneal and subcutaneous injection of the oil, sarcomas have been obtained in 2 mice and 15 rats. Because of a very high mortality rate in the early part of the experiment, mice have not proven satisfactory for this type of study, but 10 per cent of rats which received an adequate amount of treatment and survived 9 months or more developed tumors. Sediment-free oil and sediment-containing oil were both found to produce tumors, and 1 of 30 rats which received expressed oil intraperitoneally also developed sarcoma.

CARCINOGENESIS IN GUINEA PIG. MICHAEL B. SHIMKIN and G. BURROUGHS MIDER. (National Cancer Institute, Bethesda, Md.)

We are reporting the easy induction of tumors in guinea pigs following subcutaneous injection of methylcholanthrene, with special reference to the use of inbred guinea pigs. The homozygous guinea pigs used in this experi-

ment were inbred at the Beltsville Experiment Station of the Department of Agriculture since 1906. We are indebted to Doctor Orson N. Eaton for the generous supply of the animals.

Forty guinea pigs, of inbred families 2, 13, and 35, and stock guinea pigs, were injected subcutaneously with 20 to 40 mgm. of methylcholanthrene dissolved in sesame oil. The highest dose was divided into four equal fortnightly injections; the others were given as single injections.

Most of the animals of family 2 died within 15 weeks of ascites, atrophy of the spleen, and hemorrhage in the liver; this syndrome was limited to guinea pigs of this strain. Thirty-four animals survived over 18 weeks, and 29 developed tumors at the site of injection. The tumors became palpable in 18 to 40 weeks, or in an average of 25 weeks after injection. Seven guinea pigs developed 2 separate subcutaneous tumors (fibrosarcoma), and in 2 the tumors were of a mixed type. Of the 38 tumors in 29 guinea pigs, 29 were fibrosarcomas. Nine of the tumors were liposarcomas. Five of the tumors contained bone or cartilage. One of these tumors was contiguous with an eroded area in the humerus, but the remainder were extraskeletal. Four tumors, 3 fibrosarcomas and 1 liposarcoma, metastasized to the lungs. None of the metastases contained areas of bone.

Six of 7 tumors that arose in inbred guinea pigs were transplanted successfully into guinea pigs of the same family as that in which the tumor arose. No growth was obtained in animals of other families.

There were no strain differences in susceptibility, under the conditions of the experiment, and the stock guinea pigs were as susceptible as the inbred animals. Family 2 guinea pigs developed ascites and hepatic and splenic lesions, and in families 13 and 35 there was significant loss of weight with the higher dose of the hydrocarbon.

The availability of guinea pigs for cancer research is demonstrated, since homozygous animals are available and since there is a ready method of inducing subcutaneous tumors.

Published in the *Journal of the National Cancer Institute,* 1:707-725. 1941.

Discussion

SHIELDS WARREN (Boston, Mass.): This contribution by Drs. Shimkin and Mider is very valuable. The demonstration of the importance of using homozygous guinea pigs is striking.

The frequency of liposarcoma among the connective tissue tumors induced in the guinea pig is particularly noteworthy. Liposarcomas are rare in mice.

EDGAR ALLEN (New Haven, Conn.): One of the outstanding points, to my mind, in this paper by Drs. Shimkin and Mider is that the tissues which have reacted in the guinea pig to estrogens have been principally mesenchymatous. In Nelson's experiments with estrogens, the guinea pig uterus was "studded with fibromyomas." Lipschütz also reported fibromyomas. Other common laboratory animals do not react in this way to prolonged estrogenic treatment.

Negro women have a higher incidence of spontaneous uterine fibroids than whites. Is this weak point a genetic susceptibility?

CELLULAR INJURY IN RELATION TO PROLIFERATIVE AND NEOPLASTIC RESPONSE. VALY MENKIN. (Harvard University Medical School, Boston, Mass.)

Published in *Cancer Research,* 1:548-556. 1941; abstract p. 580.

THE OCCURRENCE AND PATHOLOGY OF SPONTANEOUS CARCINOMA OF THE LUNG IN MICE. H. GIDEON WELLS, MAUD SLYE, and HARRIET F. HOLMES. (University of Chicago, Chicago, Ill.)

Published in *Cancer Research,* 1:259-261. 1941; abstract p. 357.

HORMONAL CONTENT OF OVARIAN CYSTS. RUTH M. WATTS (by invitation) and FRED L. ADAIR (by invitation). (University of Chicago, Chicago, Ill.)

Published in *Cancer Research,* 1:638-648. 1941; abstract p. 682.

Discussion

E. ALLEN (New Haven, Conn.): This paper is a good illustration of the fact that it is possible to obtain fresh tissue directly from the operating table and test it for hormone content immediately without waiting for biochemical extraction.

There is now considerable experimental evidence concerning endocrine causes of ovarian cysts. It is possible to produce them experimentally by an imbalance of pituitary gonadotropic fractions. The cystic condition of the ovary produced by increased pituitary stimulation in the experimental animal is a temporary condition. For instance, van Wagenen has induced extreme cystic transformation of the ovaries by administration of pituitary gonadotropic hormone to monkeys. This condition subsides when the pituitary stimulus is withdrawn. Such animals after mating have normal periods of gestation and lactation. It seems probable that unbalanced pituitary stimulation, rather than something inherent in the ovaries, is largely responsible for the formation of cysts. The occurrence of reddening and swelling of the sexual skin in these experimental monkeys is an indication that the follicular cysts are secreting ovarian hormone. However, tests of unconcentrated fluid from such cysts in monkeys have been negative.

R. M. WATTS: I have analyzed the cystic fluid from a number of animals (rats, monkeys, and dogs) and have not yet found estrogenic hormone in any of them.

FURTHER OBSERVATIONS ON THE EFFECT OF YEAST EXTRACT ON SPONTANEOUS BREAST ADENOCARCINOMAS OF MICE. R. LEWISOHN, C. LEUCHTENBERGER (by invitation), R. LEUCHTENBERGER (by invitation), and K. BLOCH (by invitation). (Mount Sinai Hospital, New York, N. Y.)

Read by title; abstract not available.

EFFECT OF PANTOTHENIC ACID ON GROWTH OF SPONTANEOUS MAMMARY CARCINOMA IN C_3H MICE. H. P. MORRIS and S. W. LIPPINCOTT (by invitation). (National Cancer Institute, Bethesda, Md.)

Read by title; abstract not available.

PRODUCTION OF LUNG TUMORS IN STRAIN A MICE. C. F. LOOSI (by invitation) and P. E. STEINER. (University of Chicago, Chicago, Ill.)

Read by title; abstract not available.

A CRITICAL HISTOLOGIC REVIEW OF CASES SEEN AT THE NEW YORK STATE INSTITUTE FOR THE STUDY OF MALIGNANT DISEASES. E. M. BURKE and A. A. THIBAUDEAU. (New York State Institute for the Study of Malignant Diseases, Buffalo, N. Y.)

Read by title; abstract not available.

THE ACTION OF X-RAYS ON SPERM MOTILITY AND SUBSEQUENT EMBRYONIC DEVELOPMENT. P. S. HENSHAW. (National Cancer Institute, Bethesda, Md.)

Read by title; abstract not available.

American Association for Cancer Research, Inc.

34th Annual Meeting

Hotel Stevens, Chicago, Illinois, April 14-16, 1941

Proceedings of Business Sessions

Minutes of the First Meeting of the Board of Directors held April 14, 1941

The first meeting of the Board of Directors of the American Association for Cancer Research, Inc., a New York corporation, was held at Hotel Stevens, City of Chicago, State of Illinois, on the 14th day of April, 1941, at 7:35 o'clock in the afternoon, pursuant to a written consent and waiver of notice thereof signed by all of the directors of the Corporation, fixing the time and place thereof. There were present: Messrs. Simpson, Voegtlin, Bell, Bagg, Smith, Clowes, Andervont, Woglom, Little, Thibaudeau, and Murphy, constituting a quorum.

Dr. B. T. Simpson was chosen temporary Chairman of the meeting and Dr. Thibaudeau was appointed Secretary of the meeting and kept the minutes.

The Chairman then submitted to the meeting a form of By-Laws for the regulation of the affairs of the Corporation. After a full discussion of the provisions of each article of the By-Laws, said By-Laws were unanimously adopted as and for the By-Laws of the Corporation, and the Secretary was instructed to cause the same to be inserted in the minute book immediately following the copy of the Certificate of Incorporation.

The Chairman stated that the By-Laws provided that the Directors should divide themselves into three classes, and that the first of such classes should serve until the first annual meeting of members, the second class until the second annual meeting, and the third class until the third annual meeting.

After discussion, on motion duly made and seconded, it was unanimously

RESOLVED, that Drs. Bell, Clowes, Little, and Woglom shall constitute the first class of directors; that Drs. Ewing, Murphy, Smith, and Voegtlin shall constitute the second class of directors; and that Drs. Andervont, Bagg, Simpson, and Thibaudeau shall constitute the third class of directors.

The Chairman then stated that it was provided in the By-Laws that the Board of Directors should nominate candidates for President, Vice-President, and Secretary-Treasurer of the Corporation.

After discussion, on motion duly made and seconded it was unanimously

RESOLVED, that the following persons be, and they hereby are, nominated for President, Vice-President, and Secretary-Treasurer of the Corporation:

Dr. Carl Voegtlin, *President*
Dr. Shields Warren, *Vice-President*
Dr. A. A. Thibaudeau, *Secretary-Treasurer*

Reports of Officers—None.

Reports of Committees—

1. PROGRAM—Dr. Smith, *Chairman,* reported on the difficulties of arranging the program with the unusual number of papers and suggested the desirability of having titles submitted earlier. The Program Committee should consist of at least three members. Requests for time on the program should be accompanied by a comprehensive abstract.
2. JOURNAL—Dr. Little, *Chairman,* reported on the establishment of the new journal, *Cancer Research,* which is to be the official organ of the Association. The International Cancer Research Foundation, The Anna Fuller Fund, and The Jane Coffin Childs Memorial Fund for Medical Research have collaborated in the foundation and support of the journal. The journal has been aided also by a gift of $1,000.00 from the Eli Lilly Company. It was strongly urged that each member of the Association subscribe to the journal in order that it may become financially self-supporting.
3. NOMINATION—Dr. Bagg, *Chairman,* reported on the activities of this committee. Some discussion as to the activities of branch societies took place. Dr. Bagg reported on the meetings of the Central-Eastern branch (Round Table for Cancer Research) with organization meeting in Philadelphia in October and subsequent meetings in Washington and New York. Activities of the Western New York branch were reported by Dr. Thibaudeau and of the Minnesota branch by Dr. Bell.

A vote of thanks was moved and seconded to the members of the firm of Root, Clark, Buckner and Ballantine who have, without charge, assisted in the preparation of Articles of Incorporation and of the Constitution and By-Laws of the Association.—Carried.

The Secretary was ordered to convey to the management of the Hotel Stevens its appreciation for courtesies extended.

The financial report of the Treasurer was read and accepted.

The Secretary was authorized to expend not more than $100.00 for secretarial services.

It was moved and carried that the Secretary request the journal, *Cancer Research,* to publish the Constitution

and By-Laws in an early number and to order 600 reprints for distribution to members.

The Secretary-Treasurer was authorized to transfer the funds in the checking account of the Association to a bank in the community in which he resides.

Ninety new members were elected to membership.

There being no further business to come before the meeting, it was, on motion duly made and seconded, unanimously

RESOLVED, to adjourn.

Adjourned, 11:30 P.M.

B. T. Simpson, *Chairman*

A. A. Thibaudeau, *Secretary*

Minutes of Meeting of Incorporators and Members held April 15, 1941

The meeting of incorporators and members was held at Hotel Stevens, in the City of Chicago, State of Illinois, on the 15th day of April, 1941 at 9:00 o'clock in the forenoon.

Dr. Burton T. Simpson, one of the incorporators, acted as Chairman of the meeting, and Dr. Thibaudeau acted as Secretary of the meeting and kept the minutes.

The Secretary presented a notice of the meeting which had been mailed to each member of the Corporation at his address as it appeared upon the records of the Corporation.

The Secretary of the meeting submitted a list of the members present in person or by proxy. The total number of members shown on such list amounted to 166. The Secretary was directed to file such list with the minutes of the meeting.

The Secretary presented and read to the meeting a copy of the Certificate of Incorporation and reported that it had been filed in the office of the Department of State of the State of New York on the 9th day of April, 1940. The Secretary was directed to cause a copy of such certificate to be prefixed to the minutes of the meeting.

The Secretary presented and read a form of By-Laws for the regulation of the affairs of the Corporation. The Chairman stated that the By-Laws were in exactly the same form as the By-Laws which had been submitted to the meeting of the members of the American Association for Cancer Research on March 19 and 20, 1940, except that certain changes, including the changes recommended at such meeting, had been made. The Chairman further stated that Sections 1 and 5 of Article One, subsections (a), (b), (f), and (i) of Section 2 and Sections 4 and 6 of Article Two, Sections 1 and 2 of Article Three, and Section 5 of the present Article Four had been altered and explained the nature of the changes. The Chairman also pointed out that former Article Four had been omitted.

After discussion, on motion duly made, seconded, and carried, said By-Laws were unanimously adopted as and for the By-Laws of the Corporation, and the Secretary was directed to file a copy of such By-Laws in the minute book immediately following the copy of the Certificate of Incorporation.

The Chairman then stated that the following persons had been named as directors in the Certificate of Incorporation: Drs. Howard B. Andervont, Halsey J. Bagg, Elexious T. Bell, George H. A. Clowes, James Ewing, Clarence C. Little, James B. Murphy, Burton T. Simpson, George M. Smith, Alphonse Thibaudeau, Carl Voegtlin, and William H. Woglom.

The Chairman stated that he had been advised that in accordance with the provisions of the By-Laws, such directors had divided themselves into three classes, the first class to serve until the first annual meeting of members, the second class, until the second annual meeting of members, and the third class, until the third annual meeting of members. The Chairman stated that Drs. Bell, Clowes, Little, and Woglom were in the first class; Drs. Ewing, Murphy, Smith, and Voegtlin in the second class, and Drs. Andervont, Bagg, Simpson, and Thibaudeau in the third class. The Chairman stated that it would be advisable to proceed with the election of four directors, to serve a term of three years until the election of their successors, to take the place of the four directors of the first class whose term expired with the first annual meeting of the members of the Corporation. The Chairman pointed out that in the notice of meeting sent to each member, there had been included a ballot for the election of four directors to take the place of the four directors in the first class. The Chairman further stated that of the ballots received by mail from members, 133 votes had been cast for Dr. Shields Warren, as Director of the Corporation, 128 votes had been cast for Dr. Bayne-Jones, as Director of the Corporation, 107 votes had been cast for Dr. Little, as Director of the Corporation, and 90 votes had been cast for Dr. Woglom, as Director of the Corporation and that no other person had received as many votes as Dr. Woglom.

It was moved and seconded that these members be elected Directors.—Carried.

The Chairman stated that it seemed advisable to proceed with the election of the President, Vice-President, and Secretary-Treasurer of the Corporation. He stated that the By-Laws provided that the Board of Directors should nominate candidates for President, Vice-President, and Secretary-Treasurer of the Corporation, and that at the meeting of the Board of Directors held on April 14, 1941, the Board of Directors had nominated Dr. Carl Voegtlin for President, Dr. Shields Warren for Vice-President, and Dr. A. A. Thibaudeau for Secretary-Treasurer of the Corporation. The Chairman pointed out, however, that the By-Laws also provide that the officers shall be elected either from the candidates nominated by the Board of Directors or from candidates nominated by the members and called for nominations. Nominations having been made, the following officers were unanimously elected:

Dr. C. Voegtlin, *President*

Dr. S. Warren, *Vice-President*

Dr. A. A. Thibaudeau, *Secretary-Treasurer*

There being no further business to come before the meeting, it was, on motion duly made and seconded, unanimously

RESOLVED, to adjourn.

Adjourned

B. T. Simpson, *Chairman*

A. A. Thibaudeau, *Secretary*

The meeting then proceeded with the Scientific Program.

Minutes of a Special Meeting of the Board of Directors held April 16, 1941

A special meeting of the Board of Directors of the American Association for Cancer Research, Inc., a New York corporation, was held at Hotel Stevens, City of Chicago, State of Illinois, on the 16th day of April, 1941, at 12:15 o'clock in the afternoon, pursuant to a written consent and waiver of notice thereof signed by all of the directors of the Corporation, fixing the time and place thereof. There were present: Messrs. Voegtlin, Little, Murphy, Simpson, Woglom, Bayne-Jones, Andervont, Bagg, Thibaudeau, and Warren, constituting a quorum.

Dr. Voegtlin was chosen temporary Chairman of the meeting and Dr. Thibaudeau was appointed Secretary of the meeting and kept the minutes.

Waiver of Notice of a Special Meeting of Board of Directors to be held April 16, 1941.—We, the undersigned, being all of the directors of American Association for Cancer Research, Inc., a corporation organized and existing under the laws of the State of New York, do hereby severally waive notice of the time, place, and purposes of a special meeting of the Board of Directors of said Corporation, and we do hereby consent that the same be held at Hotel Stevens, in the City of Chicago, State of Illinois, on the 16th day of April, 1941, at 12:15 o'clock in the afternoon.

And we do hereby severally waive all requirements of the statutes of the State of New York and of the By-Laws of said Corporation as to notice of said meeting, and we do hereby consent to the transaction at said meeting of any and all business which may properly come before it, or any adjournment or adjournments thereof, including, but without limiting the generality of the foregoing, the following:

(1) Approval and adoption of the corporate seal of the Corporation;
(2) Approval of minutes of meeting of incorporators and members;
(3) Adoption of banking resolutions; and
(4) Designation of principal office of the Corporation in New York.

Dated April 16, 1941.

Carl Voegtlin
A. A. Thibaudeau
Halsey J. Bagg
H. B. Andervont
Stanhope Bayne-Jones
Wm. H. Woglom
Shields Warren
Burton T. Simpson
Jas. B. Murphy
Clarence C. Little

The Chairman presented to the meeting an impression of the common or corporate seal of the Corporation in the following form: (impression of corporate seal)

Upon motion duly made and seconded, the following resolution was unanimously adopted:

RESOLVED, that the form of the seal which has been submitted to this meeting, an impression of which is attached hereto, in words and figures as follows:

"American Association for Cancer
Research, Inc.
Corporate Seal 1940
New York"

be, and it hereby is, approved and adopted as the common or corporate seal of this Corporation.

The Chairman then submitted to the meeting a copy of the minutes of the meeting of the incorporators and members. Such minutes were, after discussion, unanimously approved and ratified.

After discussion, on motion duly made and seconded, it was unanimously

RESOLVED, that A. A. Thibaudeau, and Burton T. Simpson, be and each of them hereby is, authorized, in the name and on behalf of the Corporation to open a bank account or bank accounts with such banks, bankers and/or trust companies as they or each of them shall determine, and to deposit therein to the credit of the Corporation from time to time any and all monies and checks of the Corporation; and

RESOLVED, that the banks, bankers and/or trust companies so designated as depositories of the Corporation be, and they hereby are, severally authorized to honor and pay all checks, drafts and other orders for the payment of money drawn upon such account or accounts (including checks, drafts or other orders for the payment of money payable to the order of one or both of the persons making, signing or drawing them) made, signed or drawn by the following persons: A. A. Thibaudeau or Burton T. Simpson.

The Chairman stated that it would be advisable to adopt a resolution fixing the principal office of the Corporation in New York.

After discussion, on motion duly made and seconded it was unanimously

RESOLVED, that in compliance with the laws of the State of New York and the Certificate of Incorporation of the Corporation, the principal office of the Corporation in the State of New York be established and maintained at 113 High Street, in the City of Buffalo, County of Erie, State of New York, and that books of account of the Corporation be kept at said office.

It was duly moved and seconded that the President be empowered to appoint committees on Program and the Journal.—Carried.

Dr. Voegtlin thereupon appointed the following committees:

PROGRAM—C. C. Little, *Chairman,* Jas. B. Murphy, Shields Warren.

JOURNAL—Jas. B. Murphy, *Chairman,* S. Bayne-Jones, George M. Smith, C. C. Little.

It was duly moved and seconded that the President appoint a membership committee.—Carried.

Dr. Voegtlin appointed Dr. W. H. Woglom Chairman of the Membership Committee with Dr. S. Bayne-Jones and H. B. Andervont.

After some discussion on the Pan-American Union against Cancer, it was unanimously resolved that, in view of published information received from the United States Government, the Board of Directors does not confirm the action of its President in the appointment of a representative to the Pan-American Union against Cancer.

After discussion, on motion made and duly seconded, it was decided to appoint a committee for the investigation of carcinogenic substances used in the preparation of foods.

Dr. G. H. A. Clowes was appointed Chairman of the committee to consult with the Food and Drug Administration of the Social Security Agency, in reference to plans for testing drugs in common use or compounds incorporated into foods as to their possible carcinogenic activity. Dr. Clowes was empowered to form such a committee consisting of members of the Association.

There being no further business to come before the meeting, it was, on motion duly made and seconded, unanimously

RESOLVED to adjourn.

Adjourned

CARL VOEGTLIN, *Chairman*

A. A. THIBAUDEAU, *Secretary*

AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

BY-LAWS

ARTICLE I

Section 1. Members. There shall be seven classes of members as follows:

(a) Donors—who shall give to the Corporation the sum of $1,000 or more each. Donors shall be members for life without dues.

(b) Life Members—who shall give to the Corporation the sum of $100. Life members shall be members for life without dues.

(c) Patrons—who shall pay dues to the Corporation of $50 per annum.

(d) Sustaining Members—who shall pay dues to the Corporation of $25 per annum.

(e) Contributing Members—who shall pay dues to the Corporation of $10 per annum.

(f) Active Members—who shall day dues to the Corporation of $2.00 per anum.

(g) Honorary Members—who need not pay dues to the Corporation.

All persons who, on the date of incorporation of the Corporation, were active members in good standing of the American Association for Cancer Research, an incorporated association, shall become Active Members of the Corporation as of such date.

All persons who, on the date of incorporation of the Corporation, were Honorary Members of said American Association for Cancer Research shall become Honorary Members of the Corporation as of such date, without payment of dues.

Donors, Life Members, Patrons, Sustaining Members, Contributing Members and Active Members shall have equal privileges of membership and each shall be entitled to one vote at all meetings of members.

Section 2. Election of Members. The Board of Directors at any time, and from time to time, may elect to membership such persons as they consider eligible, except that Honorary Members may be elected by it only if recommended by the Executive Committee.

Section 3. Meetings of Members.

(a) The annual meeting of members shall be held at such time and place, within or without the State of New York, as may be designated in the notice of the meeting.

(b) Special meetings of members may be called by the President or, in his absence or disability, by the Vice-President, only upon (a) the request of the Board of Directors or (b) the request of thirty or more members. Special meetings shall be held at such time and place within or without the State of New York as may be designated in the notice of the meeting.

(c) Notice of each meeting of members, whether annual or special, shall be printed or written, signed by an executive officer, and mailed to each member at his address as it appears upon the books or records of the Corporation not less than ten days nor more than forty days prior to the date of the meeting, and the notice of each special meeting shall state the purpose or purposes for which it is to be held.

(d) A quorum of members shall consist of not less than one-third of the total number of members; provided, however, that if there shall be twenty-seven or more members of the Corporation, a quorum shall consist of nine.

Section 4. Resignations. Resignations of members shall be in writing addressed to the Board of Directors, but no resignation shall be accepted or take effect if the member presenting it shall be indebted to the Corporation.

Section 5. Expulsion. Any member who fails or neglects to pay his dues, or whose conduct shall in the opinion of the Board of Directors be prejudicial to the welfare of the Corporation, may be dropped from membership by the Board of Directors; provided, however, that notice in writing shall be given to such member of the action contemplated and of the grounds therefor at least 15 days previous to the date for the proposed expulsion of such member, and that such member shall be given an opportunity to present in written form and/or by personal appearance before the Board of Directors a defense of the act or acts upon which the proposed expulsion is based.

Section 6. Transfers of Membership. Memberships may be transferred by the Executive Committee from one class to another because of changes in the sum paid annually.

Article II

Section 1. The Board of Directors. The number of directors shall be twelve, who shall be members and who shall divide themselves into three classes. The first class shall serve until the first annual meeting of members, the second class shall serve until the second annual meeting of members, and the third class shall serve until the third annual meeting of members. Directors shall be elected at each annual meeting of members to succeed the directors whose terms shall have expired, and each director then chosen shall serve for a term of three years and until the election of his successor.

Section 2. Duties of Board of Directors. The Board of Directors shall have the following powers and duties:

(a) To nominate candidates for President, Vice-President, and Secretary-Treasurer of the Corporation.

(b) To fill vacancies that may from time to time occur among the officers.

(c) To direct and supervise the activities of the Corporation, its representatives and employees.

(d) To appoint such committees, standing and special, as may be necessary or desirable to carry on the work of the Corporation.

(e) To prepare or cause to be prepared and to approve annually a budget for the ensuing fiscal year.

(f) To supervise, either directly or through a duly appointed committee, the investment and disbursement of all funds of the Corporation.

(g) To fix the compensation of all representatives and employees of the Corporation.

(h) To present at the annual meeting of members a report as provided by Section 46 of the Membership Corporations Law.

(i) To elect such other officers as may be necessary properly to carry on the work of the Corporation.

(j) To perform such other duties as may be required by law.

Section 3. Annual Meetings. The Board of Directors shall meet immediately after each annual meeting of members in such place, within or without the State of New York, as may be designated in the notice of the meeting.

Section 4. Special Meetings. Special meetings of the Board of Directors may be called by the President or, in his absence or disability, by the Vice-President, and shall be called by him at the request of any two members of the Board of Directors. Special meetings may be held at such time or place, within or without the State of New York, as may be designated in the notice of the meeting. Notice of special meetings shall be given by mail, telephone, telegram, or personally, and shall be so given at least four days before the date of the meeting.

Section 5. Quorum. A quorum of the Board of Directors shall consist of five of its members.

Section 6. Vacancies. Any vacancy or vacancies in the Board of Directors however caused may be filled by a majority of the remaining members of the Board of Directors then in office, though a majority of such remaining members do not constitute a quorum. The director chosen to fill such a vacancy shall hold office only for the unexpired portion, if any, of the term of the director whose office shall have become vacant.

Article III

Section 1. Officers. The officers of the Corporation shall be a President, a Vice-President, and a Secretary-Treasurer selected from the Board of Directors. The officers shall be elected by the members either from the candidates nominated by the Board of Directors or from candidates nominated by the members. Each officer shall hold office until the next annual meeting of the members and until his successor shall have been elected and shall have qualified.

The Board of Directors may appoint other officers, including an additional Vice-President and an Assistant Secretary-Treasurer to serve for such terms and perform such duties as the Board of Directors may prescribe.

Section 2. Duties of Officers.

President. The President shall be the chief executive of the Corporation and shall preside at all meetings of the Board of Directors.

Vice-President. The Vice-President, in the absence or during the disability of the President, shall have all the powers and perform all the duties of the President.

Secretary-Treasurer. The Secretary-Treasurer shall have the care and custody of all funds and securities and valuable documents belonging to the Corporation. The Secretary-Treasurer shall deposit the funds of the Corporation to its credit in such banks, trust companies or depositaries as the Board of Directors, or the Committee appointed by the Board of Directors to supervise the investment and disbursement of the funds of the Corporation, may designate. The Secretary-Treasurer shall sign all checks and drafts in the name of the Corporation. The Secretary-Treasurer shall keep full books of account of the Corporation and shall render to the Board of Directors from time to time at its request, and annually to the members, a full statement in regard to its funds.

The Secretary-Treasurer shall keep all minutes and proceedings of the members and of the Board of Directors in books kept for that purpose. He shall give notice of all meetings of the members of the Board of Directors. He shall have the custody of the seal of the Corporation and shall affix the same when authorized to do so by the Board of Directors.

Article IV

General

Section 1. Fiscal Year. The fiscal year of the Corporation shall begin on the first day of January in each year and shall end on the following 31st day of December.

Section 2. Dues. Dues shall be payable in advance on or before the first day of March, in each year.

Section 3. Local Work. Branches of the Corporation may be formed in the name of the Corporation in such areas

as may be approved by the Board of Directors, to carry on work in conjunction with the Corporation under the direction and subject to the approval of the Board of Directors of the Corporation.

Section 4. Seal. The seal of the Corporation shall be circular in form with the words, "American Association for Cancer Research, Inc." on the circumference and the following words in the center:

Corporate Seal 1940 New York.

Section 5. Order of Business. At all regular meetings of members and of the Board of Directors, the order of business shall be as follows:

1. Roll call.
2. Reading of minutes.
3. Reports of officers.
4. Reports of standing committees.
5. Reports of special committees.
6. Reports of other representatives of the Corporation.
7. Unfinished business.
8. New business.
9. Adjournment.

At every annual meeting of members, the directors, after the reading of the minutes, shall submit a report as required by Section 46 of the Membership Corporations Law.

Section 6. Execution of Instruments. All formal agreements shall be signed by the President or the Vice-President or the Secretary-Treasurer unless otherwise ordered by the Board of Directors.

Article V

Section 1. Amendments to By-Laws. These By-Laws may be amended, added to, or repealed without notice at any annual meeting or with notice at any special meeting of members by a two-thirds vote of the members present or represented by proxy, or without notice at any annual meeting or with notice at any special meeting of the Board of Directors by a two-thirds vote of the directors present, as the case may be. Any amendment made by the Board of Directors shall be reported to the members at the next annual meeting.

American Association for Cancer Research, Inc.

List of Members

1941

A

Abels, Jules C., M.D., Memorial Hospital, 444 E. 68th St., New York, N. Y.
Ackerman, Lauren V., M.D., Ellis Fischel State Cancer Hospital, Highway 40 and N. Garth Ave., Columbia, Mo.
Adair, Frank E., Sc.D., M.D., 75 E. 71st St., New York, N. Y.
Allen, Edgar, Ph.D., Sc.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Amolsch, Arthur L., M.D., Wayne University College of Medicine, 1512 St. Antoine St., Detroit, Mich.
Anderson, Rudolph J., Ph.D., 101 Cottage St., New Haven, Conn.
Andervont, Howard B., Sc.D., National Cancer Institute, Bethesda, Md.
Apfelbach, Carl W., M.D., Rush Medical College, 1758 W. Harrison St., Chicago, Ill.
Arons, Isidore A., M.D., 57 W. 57th St., New York, N. Y.
Aub, Joseph C., M.D., Collis P. Huntington Memorial Hospital, 695 Huntington Ave., Boston, Mass.

B

Bachmann, Werner E., Ph.D., Department of Chemistry, University of Michigan, Ann Arbor, Mich.
Bagg, Halsey J., Ph.D., Memorial Hospital, 444 E. 68th St., New York, N. Y.
Bailey, Percival, Ph.D., M.D., University of Illinois College of Medicine, 1853 W. Polk St., Chicago, Ill.
Baker, Abe B., M.D., 3500 Dupont Ave. S., Minneapolis, Minn.
Ball, Howard A., M.D., 233 A St., San Diego, Calif.
Ballantyne, Elliott N., M.D., St. Joseph's Hospital, 316 John St. S., Hamilton, Ontario, Canada.
Barrett, Morris K., M.D., Rockefeller Institute for Medical Research, 66th St. and York Ave., New York, N. Y.
Barron, Moses, M.D., 1127 Medical Arts Building, Minneapolis, Minn.
Baumann, Carl A., Ph.D., Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wis.
Bayne-Jones, Stanhope, M.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Beard, Howard H., Ph.D., Louisiana State University School of Medicine, 1542 Tulane Ave., New Orleans, La.
Beck, Frances F., Ph.D., 215 Northway, Guilford, Baltimore, Md.
Behan, Richard J., M.D., 500 Penn Ave., Pittsburgh, Pa.
Belkin, Morris, Ph.D., Yale University School of Medicine, 333 Cedar Street, New Haven, Conn.
Bell, Elexious T., M.D., University of Minnesota Medical School, Minneapolis, Minn.
Berdez, George L., M.D., St. Mary's Hospital, 5th Ave. E., and 3rd St., Duluth, Minn.
Bergmann, Werner, Ph.D., 44 Cottage St., New Haven, Conn.
Berrill, Norman J., Ph.D., Sc.D., Department of Zoology, McGill University, Montreal, Canada.
Berry, George P., M.D., University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y.
Bischoff, Fritz E., Ph.D., Santa Barbara Cottage Hospital, 320 W. Pueblo St., Santa Barbara, Calif.
Bishop, Everett L., M.D., Albert Steiner Clinic for Cancer and Allied Diseases, 384 Peachtree St. N.E., Atlanta, Ga.
Bittner, John J., Ph.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.
Blady, John V., M.D., 3213 N. 17th St., Philadelphia, Pa.
Bleyer, Leo F., M.D., Arnot Ogden Memorial Hospital, Roe Ave. and Grove St., Elmira, N. Y.
Bloor, Walter R., Ph.D., University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y.
Blum, Harold F., Ph.D., National Cancer Institute, Bethesda, Md.
Blumenthal, Herman T., Ph.D., Washington University School of Medicine, 640 S. Kingshighway, St. Louis, Mo.
Branch, Charles F., M.D., Boston University School of Medicine, 80 E. Concord St., Boston, Mass.
Breslich, Paul J., M.D., Northwest Clinic, Minot, N. D.
Briggs, Robert W., Ph.D., Department of Zoology, McGill University, Montreal, Canada.
Brown, Samuel, M.D., University of Cincinnati, 707 Race St., Cincinnati, Ohio.
Brues, Austin M., M.D., Collis P. Huntington Memorial Hospital, 695 Huntington Ave., Boston, Mass.
Brunschwig, Alexander, M.D., University of Chicago School of Medicine, 950 E. 59th St., Chicago, Ill.
Bryan, W. Ray, Ph.D., National Cancer Institute, Bethesda, Md.
Buchwald, Kenneth, A.M., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Bugher, John C., M.D., Rockefeller Foundation, Apartado 2508, Bogota, Colombia, South America.
Bunting, Charles H., M.D., University of Wisconsin Medical School, 408 N. Charter St., Madison, Wis.
Burk, Dean, Ph.D., Cornell University Medical College, 1300 York Ave., New York, N. Y.
Burke, Eugene M., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Burn, Caspar G., M.D., 200 E. 18th St., Brooklyn, N. Y.
Burnam, Curtis F., M.D., Howard A. Kelley Hospital, Inc., 1418 Eutaw Pl., Baltimore, Md.
Burns, Edward L., M.D., Louisiana State University School of Medicine, 1542 Tulane Ave., New Orleans, La.
Burr, Harold S., Ph.D., 37 Loomis Pl., New Haven, Conn.
Burrows, Montrose T., M.D., 94 N. Madison Ave., Pasadena, Calif.

C

Callender, Col. George R., M.D., Army Medical Center, Washington, D. C.
Casey, Albert E., M.D., Louisiana State University School of Medicine, 1542 Tulane Ave., New Orleans, La.
Chambers, Robert, Ph.D., 171 E. 64th St., New York, N. Y.
Chambers, William H., Ph.D., Cornell University Medical College, 1300 York Ave., New York, N. Y.
Childs, S. Winston, Jr., 1 Wall St., New York, N. Y.
Childs, Starling W., 1 Wall St., New York, N. Y.
Christen, Henrietta C., M.D., 44 Sage Ave., Buffalo, N. Y.
Christian, Mrs. George C., 2303 Third Ave., Minneapolis, Minn.
Christy, Christ J., M.D., 361 Delaware Ave., Buffalo, N. Y.
Churchill, Herman R., D.D.S., Evans Institute, University of Pennsylvania, 40th and Spruce Sts., Philadelphia, Pa.
Claude, Albert, M.D., Rockefeller Institute for Medical Research, 66th St. and York Ave., New York, N. Y.
Clawson, Benjamin J., Ph.D., M.D., 500 S.E. Delaware St., Minneapolis, Minn.

CLOUDMAN, ARTHUR M., PH.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.
CLOWES, GEORGE H. A., PH.D., SC.D., Eli Lilly and Company, Indianapolis, Ind.
COCA, ARTHUR F., M.D., Pearl River, N. Y.
COHEN, MORTIMER, M.D., 5615 Bartlett St., Pittsburgh, Pa.
COLEY, BRADLEY L., M.D., 140 E. 54th St., New York, N. Y.
COLLIP, JAMES B., PH.D., SC.D., M.D., McGill University Faculty of Medicine, 3640 University St., Montreal, Canada.
CONNOR, CHARLES L., M.D., University of California, Medical Center, San Francisco, Calif.
COOPER, ZOLA K., PH.D., Washington University School of Medicine, Euclid Ave. and Kingshighway, St. Louis, Mo.
COWDRY, EDMUND V., M.D., Barnard Free Skin and Cancer Hospital, Washington and Theresa Aves., St. Louis, Mo.
CRAIG, FRANCIS N., PH.D., Thorndyke Memorial Laboratory, Boston City Hospital, 818 Harrison Ave., Boston, Mass.
CRAMER, WILLIAM, PH.D., SC.D., M.R.C.S., Barnard Free Skin and Cancer Hospital, Washington and Theresa Aves., St. Louis, Mo.
CRAVER, LLOYD F., M.D., 106 E. 85th St., New York, N. Y.
CRAWFORD, BAXTER L., M.D., Kent Rd. and Maple Ave., Wyncote, Pa.
CREECH, HUGH J., PH.D., University of Maryland, College Park, Md.
CREEVY, CHARLES D., PH.D., M.D., 4737 11th Ave. S., Minneapolis, Minn.
CRILE, GEORGE W., PH.D., M.D., 2620 Derbyshire Rd., Cleveland Heights, Ohio.
CROWELL, BOWMAN C., M.D., American College of Surgeons, 40 E. Erie St., Chicago, Ill.
CULLEN, THOMAS S., M.D., SC.D., 20 E. Eager St., Baltimore, Md.
CUTLER, MAX, M.D., 430 N. Michigan Ave., Chicago, Ill.

D

D'AUNOY, J. RIGNEY, M.D., 2936 Esplanade Ave., New Orleans, La.
DAVENPORT, HAROLD A., M.D., Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill.
DAVIS, WILLIAM WINLAND, PH.D., Eli Lilly and Company, Indianapolis, Ind.
DEADMAN, WILLIAM J., M.D., Hamilton General Hospital, Barton St. E., Hamilton, Ontario, Canada.
DICK, GEORGE F., SC.D., M.D., University of Chicago School of Medicine, 950 E. 59th St., Chicago, Ill.
DOBRINER, KONRAD, M.D., Memorial Hospital, 444 E. 68th St., New York, N. Y.
DOISY, EDWARD A., PH.D., SC.D., 1402 S. Grand Blvd., St. Louis, Mo.
DONNER, WILLIAM H., SC.D. (HON.), Lincoln-Liberty Building, Philadelphia, Pa.
DORFMAN, RALPH I., PH.D., Western Reserve University School of Medicine, 2085 Adelbert Rd., Cleveland, Ohio.
DORRANCE, GEORGE M., M.D., 2101 Spruce St., Philadelphia, Pa.
DOWDY, ANDREW H., M.D., Strong Memorial Hospital, 260 Crittenden Blvd., Rochester, N. Y.
DOWLING, ALEXANDER S., M.D., 134 E. 1st St., Corning, N. Y.
DUFRESNE, ORIGENE, M.D., Institut du Radium, 4120 Ontario St. E., Montreal, Canada.
DUNLAP, CHARLES E., M.D., 329 Longwood Ave., Boston, Mass.
DURAN-REYNALS, FRANCISCO, M.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.

E

EARLE, WILTON R., PH.D., National Cancer Institute, Bethesda, Md.
EBERHARD, THEODORE P., M.D., State Hospital No. 1, Fulton, Mo.
EBERLY, ALBERT D., M.D., Mt. Morris Tuberculosis Hospital, Mt. Morris, N. Y.
EDWARDS, HAROLD G. F., M.D., 417 Medical Arts Building, Shreveport, La.
EDWARDS, JESSE E., M.D., National Cancer Institute, Bethesda, Md.
EGGERS, CARL, M.D., 850 Park Ave., New York, N. Y.
EISEN, MILTON J., M.D., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, N. Y.
EISENHARDT, LOUISE, M.D., 88 Cottage St., New Haven, Conn.
ELLER, JOSEPH J., M.D., 745 Fifth Ave., New York, N. Y.
ELSBERG, CHARLES A., M.D., 168th St. and Ft. Washington Ave., New York, N. Y.
ELTON, W. NORMAN, M.D., 875 Lafayette Ave., Buffalo, N. Y.
ERDMANN, JOHN F., M.D., 122 E. 70th St., New York, N. Y.
ERF, LOWELL A., M.D., 2538 Durant Ave., Berkeley, Calif.
ESCHELMAN, KARL F., D.D.S., M.D., 351 Linwood Ave., Buffalo, N. Y.
EWING, JAMES, SC.D., M.D., Memorial Hospital, 444 E. 68th St., New York, N. Y.

F

FALK, K. GEORGE, PH.D., 40 E. 66th St., New York, N. Y.
FARROW, JOSEPH H., M.D., 121 E. 60th St., New York, N. Y.
FEKETE, ELIZABETH, A.M., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.
FETTERMAN, GEORGE H., M.D., Walnut St., Castle Shannon, Pittsburgh, Pa.
FIESER, LOUIS F., PH.D., Converse Memorial Laboratory, Harvard University, 12 Oxford St., Cambridge, Mass.
FIGGE, FRANK, PH.D., University of Maryland, School of Medicine, Lombard and Greene Sts., Baltimore, Md.
FIROR, WARFIELD M., M.D., Johns Hopkins Hospital, N. Broadway and Monument St., Baltimore, Md.
FOOT, NATHAN C., M.D., 525 E. 68th St., New York, N. Y.
FOOTE, FRANK W., JR., M.D., Memorial Hospital, 444 E. 68th St., New York, N. Y.
FRANING, EDWARD C. G., M.D., Bank Galesburg Building, Galesburg, Ill.
FRANKS, WILLIAM R., M.B., Banting Institute, 100 College St., Toronto 5, Ontario, Canada.
FRIEDMAN, HARRY F., M.D., 270 Commonwealth Ave., Boston, Mass.
FRIEDMAN, MILTON, M.D., 153 E. 72nd St., New York, N. Y.
FURTH, JACOB, M.D., Cornell University Medical College, 1300 York Ave., New York, N. Y.

G

GALLO, JAMES S., M.D., 594 Broadway, Paterson, N. J.
GARAI, FRANK, M.D., Manhattan Eye, Ear, and Throat Hospital, 210 E. 64th St., New York, N. Y.
GARDNER, WILLIAM U., PH.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
GÁSPÁR, ISTVÁN, M.D., Rochester General Hospital, 501 W. Main St., Rochester, N. Y.
GATES, OLIVE, M.D., Collis P. Huntington Memorial Hospital, 695 Huntington Ave., Boston, Mass.
GENDREAU, JOSEPH E., M.D., Institut du Radium, 4120 Ontario St., E. Montreal, Canada.
GOLTZ, HILDA L., 955 Lafayette Ave., Buffalo, N. Y.
GRADY, HUGH G., M.D., National Cancer Institute, Bethesda, Md.
GRAUER, ROBERT C., M.D., Allegheny General Hospital, 320 E. North Ave., Pittsburgh, Pa.
GRAY, SAMUEL H., M.D., Jewish Hospital, 216 S. Kingshighway Blvd., St. Louis, Mo.
GREENE, HARRY S. N., M.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
GREENSTEIN, JESSE P., PH.D., National Cancer Institute, Bethseda, Md.
GUIMARAES, UGO C., M.D., Urugayana 104, 5° andar, Rio de Janeiro, Brazil, South America.

H

HAAGENSEN, CUSHMAN D., M.D., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, N. Y.
HALPERT, BELA, M.D., 431 Millandon St., New Orleans, La.

Hamilton, James B., Ph.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Harris, Paul, M.D., Eli Lilly and Company, Indianapolis, Ind.
Harrison, Ross G., Ph.D., Sc.D., M.D., 142 Huntington St., New Haven, Conn.
Hastings, Willard S., M.D., Jeanes Hospital, Barnes and Hartel Sts., Fox Chase, Philadelphia, Pa.
Haven, Frances L., Ph.D., University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y.
Hawkins, William B., M.D., Strong Memorial Hospital, 260 Crittenden Blvd., Rochester, N. Y.
Haythorn, Samuel R., M.D., William H. Singer Memorial Laboratory, 320 East North Ave., N.S., Pittsburgh, Pa.
Hebbel, Robert, M.D., Department of Pathology, University of Minnesota Medical School, Minneapolis, Minn.
Heiman, Helen I., M.D., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, N. Y.
Heiman, Jacob, M.D., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, N. Y.
Hektoen, Ludvig, Sc.D., M.D., Chicago Tumor Institute, 21 W. Elm St., Chicago, Ill.
Hellwig, Christian A., M.D., St. Francis Hospital, 928 N. Emporia Ave., Wichita, Kan.
Helmbold, Theodore R., M.D., 5215 Celia Pl., Pittsburgh, Pa.
Helmer, Oscar M., Ph.D., Eli Lilly and Company, Indianapolis, Ind.
Helwig, Ferdinand, M.D., St. Luke's Hospital, 44th St. and Mill Creek Parkway, Kansas City, Mo.
Hengerer, Augustus W., M.D., 71 Jewett Parkway, Buffalo, N. Y.
Henshaw, Paul S., Ph.D., National Cancer Institute, Bethesda, Md.
Herger, Charles C., M.D., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Hertzler, Arthur E., Ph.D., M.D., 327 Chestnut St., Halstead, Kan.
Heston, Walter E., Ph.D., National Cancer Institute, Bethesda, Md.
Hoffman, Frederick L., LL.D., Biochemical Research Foundation of the Franklin Institute, 1978 Sunset Blvd., San Diego, Calif.
Hoffman, Joseph G., Ph.D., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Hoffman, William J., M.D., 153 E. 72nd St., New York, N. Y.
Hollander, Lester, M.D., 631 Jenkins Building, Pittsburgh, Pa.
Holmes, Harriet F., Selborne, South Batavia Rd., Batavia, Ill.
Howe, Martha E., M.D., 30 E. 40th St., New York, N. Y.
Hudson, Leona, M.S., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Hueper, Wilhelm C., M.D., 166 Nelson Rd., Scarsdale, N. Y.

I

Ikeda, Kano, M.D., 125 W. College Ave., St. Paul, Minn.
Ill, Edgar A., M.D., 1004 Broad St., Newark, N. J.

J

Jackson, Elizabeth B., 77 Marlboro St., Boston, Mass.
Jackson, Henry, Jr., M.D., Collis P. Huntington Memorial Hospital, 695 Huntington Ave., Boston, Mass.
Jacobs, William F., M.D., 408 Richmond Ave., Buffalo, N. Y.
Jacobsen, Victor C., M.D., 51 First St., Troy, N. Y.
Jessup, David S. D., M.D., 411 W. 114th St., New York, N. Y.
Jetter, Walter, M.D., Taunton, Mass.
Jobling, James W., M.D., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, N. Y.
Jones, Benjamin F., M.D., 2226 Decatur Pl. N.W., Washington, D. C.
Jones, Donald F., Sc.D., Connecticut Agricultural Experiment Station, P.O. Box No. 1106, New Haven, Conn.
Jones, E. Elizabeth, Ph.D., Department of Zoology, Wellesley College, Wellesley, Mass.
Jones, George W., M.D., Veterans Administration Facility, Hines, Ill.
Jones, R. Norman, Ph.D., Converse Memorial Laboratory, Harvard University, 12 Oxford St., Cambridge, Mass.
Jorstad, Louis H., M.D., 3720 Washington Blvd., St. Louis, Mo.
Joseph, Barnet, M.D., 344 W. 72nd St., New York, N. Y.

K

Kaempffert, Waldemar, Ph.D., 325 West End Ave., New York, N. Y.
Kahlstrom, Samuel C., M.D., Veterans Administration Facility, Bath, N. Y.
Kaplan, Ira I., M.D., 55 E. 86th St., New York, N. Y.
Karsner, Howard T., M.D., Western Reserve University School of Medicine, 2085 Adelbert Rd., Cleveland, Ohio.
*Kast, Ludwig, M.D., Josiah Macy, Jr. Foundation, 565 Park Ave., New York, N. Y.
Kellert, Ellis, M.D., Ellis Hospital Laboratory, Nott St. and Rosa Rd., Schenectady, N. Y.
Kenney, John M., M.D., Memorial Hospital, 444 E. 68th St., New York, N. Y.
Kidd, John G., M.D., Rockefeller Institute for Medical Reesarch, 66th St. and York Ave., New York, N. Y.
Kilgore, Alson R., M.D., 490 Post St., San Francisco, Calif.
Kirschbaum, Arthur, Ph.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Kletzien, Seymour, Ph.D., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Knox, Leila C., M.D., 1160 Fifth Ave., New York, N. Y.
Kopac, Mila J. Ph.D., Washington Square College, New York University, Washington Square, New York, N. Y.
Koucky, Rudolph W., M.D., St. Mary's Hospital, 2500 S. 6th St., Minneapolis, Minn.
Kraemer, William H., M.D., 2098 duPont Building, Wilmington, Del.
Krahl, Maurice E., Ph.D., Eli Lilly and Company, Indianapolis, Ind.
Krantz, John C., Ph.D., Sc.D., 29 S. Greene St., Baltimore, Md.
Kraus, Ezra J., Ph.D., University of Chicago, 1155 E. 57th St., Chicago, Ill.
Krehbiel, Otto F., M.D., P.O. Box No. 133, Red Bank, N. J.
Kress, Louis C., M.D., 188 Ruskin Rd., Eggertsville, N. Y.
Krumbhaar, Edward B., Ph.D., M.D., University of Pennsylvania School of Medicine, 36th and Spruce Sts., Philadelphia, Pa.

L

Lambert, Robert A., M.D., Rockefeller Foundation, 49 W. 49th St., New York, N. Y.
Larsen, C. Donald, Ph.D., 4419 Maple Ave., Bethesda, Md.
LaVake, Rae T., M.D., 718 Medical Arts Building, Minneapolis, Minn.
Law, Lloyd W., Ph.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.
Lawrence, John H., M.D., Crocker Laboratory, University of California, Berkeley, Calif.
Lawson, John D., M.D., Sacramento County Hospital, Stockton Blvd., Sacramento, Calif.
Lenz, Maurice, M.D., 2736 Netherland Ave., New York, N. Y.
Leonard, Veader, M.D., 7400 York Rd., Baltimore, Md.
L'Esperance, Elise S., M.D., New York Infirmary for Women and Children, 321 E. 15th St., New York, N. Y.
Levin, Isaac, M.D., 57 W. 57th St., New York, N. Y.
Levine, Michael, Ph.D., Montefiore Hospital, 150 E. Gun Hill Rd., New York, N. Y.
Lewis, Margaret R., M.D., Wistar Institute of Anatomy and Biology, 36th St. and Woodland Ave., Philadelphia, Pa.
Lewis, Warren H., M.D., Wistar Institute of Anatomy and Biology, 36th St. and Woodland Ave., Philadelphia, Pa.
Lewisohn, Richard, M.D., 1155 Park Ave., New York, N. Y.
Lippincott, Stuart W., M.D., National Cancer Institute, Bethesda, Md.

* Deceased, August 14, 1941.

Littauer, Lucius N., 235 Fourth Ave., New York, N. Y.
Little, Clarence C., Sc.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.
Lockwood, John S., M.D., University of Pennsylvania School of Medicine, 36th and Spruce Sts., Philadelphia, Pa.
Loder, Margaret M., M.D., 926 Boston Post Road, Rye, N. Y.
Loeb, Leo, M.D., Oscar Johnson Institute, Washington University School of Medcine, 640 S. Kingshighway, St. Louis, Mo.
Long, William B., M.D., 830 Park Ave., New York, N. Y.
Lorenz, Egon, Ph.D., National Cancer Institute, Bethesda, Md.
Lucké, Balduin, M.D., University of Pennsylvania School of Medicine, 36th and Spruce Sts., Philadelphia, Pa.
Luden, Georgine, Ph.D., M.D., 1006 St. Charles St., Victoria, B. C., Canada.
Lufkin, Nathaniel H., M.D., St. Barnabas Hospital, 920 S. 7th St., Minneapolis, Minn.
Lynch, Clara, Ph.D., Rockefeller Institute for Medical Research, 66th St. and York Ave., New York, N. Y.
Lynch, Francis W., M.D., 317 Lowry Medical Arts Building, St. Paul, Minn.
Lynch, Kenneth M., M.D., Medical College of South Carolina, 16 Lucas St., Charleston, S. C.

M

MacCarty, William C., Sr., M.D., Mayo Clinic, Rochester, Minn.
MacFarlane, Catharine, M.D., 136 S. 16th St., Philadelphia, Pa.
MacNeal, Ward J., Ph.D., M.D., 82 Rockrose Pl., Forest Hills, N. Y.
Mahoney, Earle B., M.D., Strong Memorial Hospital, 260 Crittenden Blvd., Rochester, N. Y.
Mallory, Frank B., Sc.D., M.D., Boston City Hospital, 818 Harrison Ave., Boston, Mass.
Manson, Melville H., Ph.D., M.D., Bell Telephone Laboratories, 463 West St., New York, N. Y.
Marine, David, Sc.D., M.D., Montefiore Hospital, 150 E. Gun Hill Rd., New York, N. Y.
Marshak, Alfred, Ph.D., Crocker Laboratory, University of California, Berkeley, Calif.
Martin, Hayes E., M.D., 30 E. 72nd St., New York, N. Y.
Matas, Rudolph, ScD., M.D., 2255 St. Charles Ave., New Orleans, La.
Mattick, Walter L., M.D., 290 Highland Ave., Buffalo, N. Y.
Maver, Mary E., Ph.D., 1316 New Hampshire Ave. N.W., Washington, D. C.
McCartney, James S., M.D., University of Minnesota Medical School, Minneapolis, Minn.
McDonald, Ellice E., M.D., Biochemical Research Foundation of the Franklin Institute, Newark, Del.
McFarland, Joseph, M.D., University of Pennsylvania School of Medicine, 36th and Spruce Sts., Philadelphia, Pa.
Medes, Grace, Ph.D., Lankenau Hospital Research Institute, Girard and Corinthian Aves., Philadelphia, Pa.
Mendel, Bruno, M.D., Banting Institute, 100 College St., Toronto 5, Ontario, Canada.
Menke, John F., M.D., Stanford University Hospital, Clay and Webster Sts., San Francisco, Calif.
Menkin, Valy, M.D., Harvard Medical School, 25 Shattuck St., Boston, Mass.
Menten, Maud L., Ph.D., M.D., University of Pittsburgh School of Medicine, Bigelow Blvd., Pittsburgh, Pa.
Meyer, Herbert W., M.D., 170 E. 78th St., New York, N. Y.
Mider, G. Burroughs, M.D., National Cancer Institute, Bethesda, Md.
Miller, David K., M.D., Edward J. Meyer Memorial Hospital, 462 Grider St., Buffalo, N. Y.
Milliken, Seth M., M.D., 951 Madison Ave., New York, N. Y.
Minot, George R., Sc.D., M.D., Thorndyke Memorial Laboratory, Boston City Hospital, 818 Harrison Ave., Boston, Mass.
Mohs, Frederic E., M.D., 1300 University Ave., Madison, Wis.
Moore, Robert A., Ph.D., M.D., Department of Pathology, Washington University School of Medicine, Euclid Ave. and Kingshighway, St. Louis, Mo.
Moritz, Alan R., M.D., Department of Legal Medicine, Harvard Medical School, 25 Shattuck St., Boston, Mass.
Morris, Harold P., Ph.D., National Cancer Institute, Bethesda, Md.
Morton, John J., M.D., 18 Audubon St., Rochester, N. Y.
Mosher, Harris P., M.D., 127 Front St., Marblehead, Mass.
Murphy, Gibbons W., M.D., Flatiron Building, Asheville, N. C.
Murphy, James B., Sc.D., M.D., Rockefeller Institute for Medical Research, 66th St. and York Ave., New York, N. Y.
Murphy, Walter T., M.D., 126 St. James Pl., Buffalo, N. Y.
Murray, Joseph M., Ph.D., University of Maine, Orono, Me.
Murray, William S., Sc.D., Biological Station, New York State Institute for the Study of Malignant Diseases, Springville, N. Y.

N

Nagayo, Mataro, M.I.A., M.D., Japanese Foundation for Cancer Research, Nishi Sugamo Toshimaku, Tokyo, Japan.
Nakahara, Waro, Ph.D., M.D., 1141 Mejiro, Tokyo, Japan.
Nathanson, Ira T., M.D., Collis P. Huntington Memorial Hospital, 695 Huntington Ave., Boston, Mass.
Nicholas, John S., Ph.D., 170 Cold Spring St., New Haven, Conn.
Noble, John F., M.D., Ancker Hospital, 1828 Highland Parkway, St. Paul, Minn.
Nolan, Lewis E., M.D., P.O. Box No. 295, Hines, Ill.
Norris, Edgar H., M.D., 607 Lakepointe Rd., Detroit, Mich.
Noyes, Helen M., A.M., Orthopedics Laboratory, University of Illinois College of Medicine, 830 S. Wood St., Chicago, Ill.

O

Oberhelman, Harry A., M.D., 701 Columbia Ave., Oak Park, Ill.
O'Brien, Joseph P., M.D., 749 S. Park Ave., Buffalo, N. Y.
O'Brien, William A. M.D., University Hospital, University of Minnesota, 412 Delaware St. S.E., Minneapolis, Minn.
Oehlbeck, Luther W. F., M.D., Memorial General Hospital, Rhoades Ave. and College St., Kinston, N. C.
Ogden, Ralph T., M.D., 179 Allyn St., Hartford, Conn.
Olch, Isaac Y., M.D., 1908 Wilshire Blvd., Los Angeles, Calif.
Opie, Eugene L., Sc.D., M.D., Cornell University Medical College, 1300 York Ave., New York, N. Y.
Ormsby, Oliver S., M.D., 25 East Washington St., Chicago, Ill.
Orr, Clifford R., M.D., University of Buffalo School of Medicine, 24 High St., Buffalo, N. Y.
Oughterson, Ashley W., M.D., New Haven Hospital, 789 Howard Ave., New Haven, Conn.
Ower, John J., M.D., Department of Pathology, University of Alberta, Edmonton, Alberta, Canada.

P

Pack, George T., M.D., 155 E. 72nd St., New York, N. Y.
Page, Irvine H., M.D., Eli Lilly and Company, Indianapolis, Ind.
Pawling, Jesse R., M.D., 205 Trust Company Building, 100 Stone St., Watertown, N. Y.
Pearce, Louise, M.D., Rockefeller Institute for Medical Research, Princeton, N. J.
Pearson, Bjarne, M.D., 6035 Hurst St., New Orleans, La.
Pecher, Charles, M.D., Crocker Laboratory, University of Califronia, Berkley, Calif.
Pendergrass, Eugene P., M.D., University of Pennsylvania School of Medicine, 36th and Spruce Sts., Philadelphia, Pa.
Permar, Howard H., M.D., Mercy Hospital, Pride and Locust Sts., Pittsburgh, Pa.
Perry, Isabella H., M.D., 2nd and Parnassus Aves., San Francisco, Calif.
Peyton, William T., Ph.D., M.D., 64 Barton Ave. S.E., Minneapolis, Minn.
Pierce, William W., M.D., 417 Bewley Building, Lockport, N. Y.
Pincus, Gregory, Sc.D., Clark University, 950 Main St., Worcester, Mass.

Pinsonneault, Germain, M.D., Institut du Radium, 4120 Ontario St. E., Montreal, Canada.
Pitts, Herman C., M.D., 68 Brown St., Providence, R. I.
Pollia, Joseph A., M.D., 269 S. Lake St., Los Angeles, Calif.
Pomeroy, Lawrence A., M.D., 952 Hanna Building, Cleveland, Ohio.
Prigosen, Rosa E., M.D., 1469 53rd St., Brooklyn, N. Y.
Prime, Frederick, M.D., 131 E. 66th St., New York, N. Y.

Q

Queen, Frank B., M.D., Northwestern University Medical School, 303 E. Superior St., Chicago, Ill.
Quick, Douglas, M.D., 350 Park Ave., New York, N. Y.
Quigley, Daniel T., M.D., 107 S. 17th St., Omaha, Neb.

R

Reimann, Stanley P., M.D., Lankenau Hospital Research Institute, Grand and Corinthian Aves., Philadelphia, Pa.
Reinhard, Melvin C., A.M., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Rezek, Philipp, M.D., James M. Jackson Memorial Hospital, 1700 N.W. 10th Ave., Miami, Fla.
Rhoads, Cornelius P., M.D., Memorial Hospital, 444 E. 68th St., New York, N. Y.
Riker, Albert J., Ph.D., Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.
Rigler, Leo G., M.D., University Hospital, 412 Delaware St. S.E., Minneapolis, Minn.
Robinson, William L., M.D., University of Toronto Faculty of Medicine, Toronto, Canada.
Roffo, Angel H., M.D., Instituto de Medicina Experimental, 5481 Ave. San Martin, Buenos Aires, Argentina, South America.
Rous, F. Peyton, Sc.D., M.D., Rockefeller Institute for Medical Research, 66th St. and York Ave., New York, N. Y.

S

Sala, Angelo M., M.D., New York City Cancer Institute, 124 East 59th St., New York, N. Y.
Salter, William T., M.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Santa Cruz, Juan Z., M.D., University of the Philippines College of Medicine, Manila, Philippines.
Scharnagel, Isabel M., M.D., 155 E. 73rd St., New York, N. Y.
Scheele, Leonard A., M.D., National Cancer Institute, Bethesda, Md.
Schiller, Walter, M.D., Cook County Hospital, Wood and Harrison Sts., Chicago, Ill.
Schram, Mildred W. S., Ph.D., International Cancer Research Foundation, Lincoln-Liberty Building, Philadelphia, Pa.
Schreiner, Bernard F., M.D., 231 Humboldt Parkway, Buffalo, N. Y.
Schrek, Robert, M.D., Veterans Administration Facility, Hines, Ill.
Seecoff, David P., M.D., 1970 Daly Ave., Bronx, N. Y.
Seelig, Major G., M.D., 4954 Lindell Blvd., St. Louis, Mo.
Selbach, Gustav, M.D., 66 Elizabeth St., Dansville, N. Y.
Selle, Wilbur A., Ph.D., University of Texas Faculty of Medicine, 912 Ave. B, Galveston, Texas.
Semken, George H., M.D., 16 W. 85th St., New York, N. Y.
Shafer, Rudolph J., M.D., 163 E. First St., Corning, N. Y.
Shear, Murray J., Ph.D., National Cancer Institute, Bethesda, Md.
Sheehan, George L., M.D., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Shemin, David, Ph.D., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, N. Y.
Shimkin, Michael B., M.D., National Cancer Institute, Bethesda, Md.
Silberberg, Martin, M.D., New York University College of Medicine, 477 First Ave., New York, N. Y.
Simmons, Channing C., M.D., 205 Beacon St., Boston, Mass.
Simonds, James P., Ph.D., M.D., Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill.
Simpson, Burton T., M.D., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Simpson, Walter M., M.D., Miami Valley Hospital, 134 Apple St., Dayton, Ohio.
Sittenfield, Maurice J., M.D., 29 W. 74th St., New York, N. Y.
Slye, Maud, Sc.D., 5836 Drexel Ave., Chicago, Ill.
Smith, George M., M.D., Pine Orchard, Conn.
Smith, Lawrence W., M.D., Temple University School of Medicine, N. Broad and Ontario Sts., Philadelphia, Pa.
Smith, Leon H., M.D., 467 Linwood Ave., Buffalo, N. Y.
Solomon, Harold A., D.D.S., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Spencer, Roscoe R., M.D., National Cancer Institute, Bethesda, Md.
Spies, John W., M.D., University of Texas Faculty of Medicine, 912 Ave. B, Galveston, Texas.
Spies, Tom D., M.D., Cincinnati General Hospital, Burnet and Goodman Sts., Cincinnati, Ohio.
Sproul, Edith E., M.D., Presbyterian Hospital, 620 W. 168th St., New York, N. Y.
Steiner, Paul E., Ph.D., M.D., Department of Pathology, University of Chicago, 58th St. and Ellis Ave., Chicago, Ill.
Stenstrom, Annette E., M.D., University of Minnesota School of Medicine, Minneapolis, Minn.
Stenstrom, Karl W., Ph.D., University of Minnesota School of Medicine, Minneapolis, Minn.
Stevens, Rollin H., M.D., 1429 David Whitney Building, Detroit, Mich.
Stevenson, Holland N., M.D., Professional Building, New Rochelle, N. Y.
Stewart, Harold L., M.D., National Cancer Institute, Bethesda, Md.
Stout, Arthur P., M.D., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, N. Y.
Strong, Leonell C., Ph.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Sugiura, Kanematsu, A.M., D.M.Sc., Memorial Hospital, 444 E. 68th St., New York, N. Y.
Swan, John M., M.D., 457 Park Ave., Rochester, N. Y.
Syverton, Jerome T., M.D., University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y.

T

Tannenbaum, Albert, M.D., 4847 Lake Park Ave., Chicago, Ill.
Taussig, Frederick J., M.D., 3720 Washington Blvd., St. Louis, Mo.
Taylor, Howard C., Jr., M.D., 842 Park Ave., New York, N. Y.
Taylor, Kenneth, M.D., 14 Sutton Pl. S., New York, N. Y.
Thibaudeau, Alphonse A., M.D., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Thomas, Walter S., M.D., Monroe County Infirmary, 435 E. Henrietta Rd., Rochester, N. Y.
Toennies, Gerrit, Ph.D., Lankenau Hospital, Girard and Corinthian Aves., Philadelphia, Pa.
Treves, Norman, M.D., 48 E. 75th St., New York, N. Y.
Turner, Charles W., Ph.D., 203 Eckles Hall, University of Missouri, Columbia, Mo.
Turner, Floyd C., M.D., National Cancer Institute, Bethesda, Md.
Twombly, Gray H., M.D., 107 E. 85th St., New York, N. Y.
Tyzzer, Ernest E., Sc.D., M.D., 175 Water St., Wakefield, Mass.

V

Voegtlin, Carl, Ph.D., National Cancer Institute, Bethesda, Md.
Von Haam, Emmerich, M.D., Department of Pathology, Ohio State University College of Medicine, Neil and 11th Aves., Columbus, Ohio.

W

Walker, Irwin M., M.D., 653 Main St., Niagara Falls, N. Y.
Walkey, Albert E., M.D., Hamilton General Hospital, Barton St. E., Hamilton, Ontario, Canada.
Wallace, Edward W., Ph.D., M.D., 1203 Ryland Ave., Cincinnati, Ohio.
Waltman, Charles A., M.D., 78 Manhattan Ave., New York, N. Y.
Warner, Stanley G., M.A., New York State Institute for the Study of Malignant Diseases, 113 High St., Buffalo, N. Y.
Warren, Shields, M.D., New England Deaconess Hospital, 195 Pilgrim Rd., Boston, Mass.
Warren, Stafford L., M.D., 199 Barrington St., Rochester, N. Y.
Wartman, William B., M.D., Western Reserve University School of Medicine, 2085 Adelbert Rd., Cleveland, Ohio.
Wehr, William H., M.D., 329 E. Delevan Ave., Buffalo, N. Y.
Weiss, Emil, Ph.D., M.D., University of Illinois College of Medicine, 1853 Polk St., Chicago, Ill.
Weller, Carl V., M.D., University of Michigan Medical School, Ann Arbor, Mich.
Wells, Arthur H., M.D., St. Luke's Hospital, 901 E. 1st St., Duluth, Minn.
Wells, H. Gideon, Ph.D., M.D., University of Chicago School of Medicine, 58th St. and Ellis Ave., Chicago, Ill.
Wende, Reinhardt C., M.D., 232 Linwood Ave., Buffalo, N. Y.
White, Abraham, Ph.D., 48 Westwood Rd., New Haven, Conn.
White, Julius, Ph.D., National Cancer Institute, Bethesda, Md.
White, Thomas N., Ph.D., National Cancer Institute, Bethesda, Md.
Widmann, Bernard P., M.D., 250 S. 18th St., Philadelphia, Pa.
Williams, George Z., M.D., 303 Roanoke St., Richmond, Va.
Wilson, Louis B., M.D., Mayo Clinic, Rochester, Minn.
Winchell, A. Vaughn, M.D., American Oncologic Hospital, 3234 Powelton Ave., Philadelphia, Pa.
Winer, Louis H., M.D., 522 Medical Arts Building, Minneapolis, Minn.
Winternitz, Milton C., M.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Wirth, John E., M.D., U. S. Marine Hospital, Wyman Park Dr. and 31st St., Baltimore, Md.
Woglom, William H., M.D., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, N. Y.
Wolbach, Simeon B., M.D., 240 Longwood Ave., Boston, Mass.
Wolff, Jack M., Ph.D., Albany Medical College, 47 New Scotland Ave., Albany, N. Y.
Womack, Nathan A., M.D., 4927 Laclede Ave., St. Louis, Mo.
Wood, Francis C., Sc.D., M.D., St. Luke's Hospital, Amsterdam Ave. and 113th St., New York, N. Y.
Woolley, George W., Ph.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.
Wright, Arthur W., M.D., Albany Medical College, 47 New Scotland Ave., Albany, N. Y.
Wyeth, George A., M.D., Faraway Farm, Hanover, N. H.

Z

Zimmerman, Harry M., M.D., Yale University School of Medicine, 310 Cedar St., New Haven, Conn.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BRADBURY, J. T., W. E. BACHMANN, and M. G. LEWISOHN. [Univ. of Michigan, Ann Arbor, Mich.] THE PRODUCTION OF CANCER BY SOME NEW CHEMICAL COMPOUNDS. FACTORS AFFECTING THE LATENT PERIOD OF TUMOR PRODUCTION. Cancer Research, 1:685-694. 1941.

Experiments with 9,10-dimethyl-1,2-benzanthracene and several of its derivatives as well as a series of cholanthrene compounds are presented. The rate of tumor production varies with the solvent used and the route of administration. Meso-dihydrocholanthrene was carcinogenic when injected subcutaneously but not when painted on the skin. 9,10-Dimethyl-1,2-benzanthracene appears to be a more active carcinogen when applied to the skin because of its toxicity when it is injected. 4-Methyl- and 5-methylcholanthrene have a similar order of activity as 3-methylcholanthrene in the production of sarcomas.—Authors' abstract.

CARRUTHERS, C., and R. E. STOWELL. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] INFLUENCE OF HEPTALDEHYDE ON PREGNANCY IN RATS. Cancer Research, 1:724-728. 1941.

Daily intraperitoneal injection of 0.2 cc. or less of heptaldehyde in lard or in acetone induced a high percentage of resorptions of embryos before the 14th day of pregnancy in the rat. Heptaldehyde administered by stomach tube was ineffective in the rat. The rats undergoing resorption of embryos typically showed a profuse placental sign occurring 3 days earlier than normal. Although hemorrhages from and death of placental tissues were observed histologically, the exact mechanism of resorption was not evident.—Authors' abstract.

LAW, L. W., and M. LEWISOHN. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] COMPARATIVE CARCINOGENICITY OF CHOLANTHRENE DERIVATIVES. Cancer Research, 1:695-698. 1941.

A comparison of the carcinogenicity of the parent hydrocarbon cholanthrene with 3 different methylated compounds and 2 hydrogenated compounds has been investigated. No difference was found in the potencies of 3- or 4-methylcholanthrene as compared with cholanthrene as regards induction of subcutaneous tumors (sarcomas). 5-Methylcholanthrene, however, was a less potent compound both as regards incidence of induced tumors and length of latent period. The two dihydro derivatives tested did not differ significantly from the parent compounds.

Both 3-methylcholanthrene and 4-methylcholanthrene were significantly more potent in inducing lung carcinomas than the parent hydrocarbon cholanthrene. 5-Methylcholanthrene was significantly less potent than cholanthrene. Hydrogenation of the cholanthrene or 3-methylcholanthrene molecule resulted in a significant decrease in carcinogenicity of these compounds in regard to lung carcinoma induction.

It is concluded that (a) cholanthrene and its derivatives act differently as carcinogens to different tissues, (b) substitution of a methyl group may increase or may decrease carcinogenicity dependent upon the substitution position within the molecule. From the data obtained, under the conditions of this experiment, the position of methylation is a more decisive factor in influencing carcinogenesis than simply addition of a methyl group.—Authors' abstract.

LUCKÉ, B., A. K. PARPART, and R. A. RICCA. [Univ. of Pennsylvania, Philadelphia, Pa.; Princeton Univ., Princeton, N. J.; and Marine Biological Laboratory, Woods Hole, Mass.] FAILURE OF CHOLEIC ACIDS OF CARCINOGENIC HYDROCARBONS TO ALTER PERMEABILITY OF MARINE EGGS AND OF MAMMALIAN ERYTHROCYTES. Cancer Research, 1:709-713. 1941.

Several types of cells which permit quantitative study of permeability (egg cells of *Arbacia* and of *Chaetopterus*, erythrocytes of mouse and beef) were exposed to choleic acid compounds of carcinogenic and related noncarcinogenic hydrocarbons; permeability of the cells to water and to a variety of solutes was then measured. The carcinogens chosen for these experiments were choleic acid compounds of 20-methylcholanthrene, 1,2,5,6-dibenzanthracene, 10-methyl-1,2-benzanthracene; the related noncarcinogens were similar compounds of phenanthrene, 1,2-benzanthracene, and acetnaphthene.

The results of the determinations showed that neither the cells exposed to the choleic acids of these carcinogens nor those exposed to related noncarcinogens differed in their permeability from the control unexposed cells. This was true for both marine eggs and erythrocytes.

Under similar experimental conditions the carcinogens did, however, induce decided disturbances of cell cleavage (using *Arbacia* eggs as the test objects).—Authors' summary.

Genetics

COLE, R. K. [Cornell Univ., Ithaca, N. Y.] GENETIC RESISTANCE TO A TRANSMISSIBLE SARCOMA IN THE FOWL. Cancer Research, 1:714-720. 1941.

These experiments which involved more than 2,600 pedigreed chicks were designed to determine the extent to which resistance to a transmissible spindle cell sarcoma (Jungherr sarcoma) could be increased by selective breeding. The breeding birds were selected on the basis of the progeny and/or sib tests. Two lines of birds were established, one characterized by a relatively high incidence of susceptibility (susceptible line) and the other by a low susceptibility (resistant line) to the sarcoma. After four generations of selection, susceptibility was increased from 70.6% in the unselected population to 77.2%. At the same time it was lowered in three generations to 12.5% in the line selected for resistance.

Susceptibility to the transmissible sarcoma is not influenced by sex nor by the age of the host. Resistance to this particular tumor is not indicative of resistance to the more common disease-producing agents affecting the fowl.—Author's abstract.

Biochemistry and Nutrition—Chemotherapy

ADAMSTONE, F. B. [Univ. of Illinois, Urbana, Ill.] RETICULUM CELL SARCOMA FOLLOWING ULCERATION OF THE INTESTINE IN VITAMIN E DEFICIENT CHICKS. Arch. Path., 31:717-721. 1941.

In a considerable proportion of chicks reared on an iron-treated, vitamin E-deficient ration, ulcers developed in the lower part of the intestine, followed by extensive tumor growths derived from the reticular connective tissue. Occasionally they penetrate the wall of the intestine, but apparently remote metastasis was not observed. They appeared only in those groups of chicks which had a vitamin A and D supplement in the form of cod liver oil or sardine oil in their diet. It remains to be seen whether the development of these tumors is due to the lack of some necessary substance in these oils or whether there is a disturbance in the utilization of vitamin D supplied in the form of cod liver oil or sardine oil under conditions of vitamin E deficiency.—H. G. W.

ADAMSTONE, F. B. [Univ. of Illinois, Urbana, Ill.] RELATION OF VITAMIN E TO SUBSTANCES OF THE ANTHRACENE GROUP. Arch. Path., 31:722-730. 1941.

A general survey of reactions to vitamin E deficiency discloses that the structures affected are all more or less intimately associated with substances of the anthracene group. This suggests that vitamin E is concerned with normal metabolism of some of the anthracene compounds. —H. G. W.

ERF, L. A., and G. FRIEDLANDER. [Univ. of California, Berkeley, Calif.] PHOSPHORUS EXCHANGE IN TISSUES OF PATIENTS WITH LYMPHOID LEUKEMIA. Proc. Soc. Exper. Biol. & Med., 47:134-136. 1941.

Radiophosphorus (P^{32}) produced by cyclotron was converted into sodium phosphate for oral and intravenous therapy for leukemic patients. Tissue pieces (about 5 gm.) obtained at autopsy from 4 patients so treated were ashed in crucibles at 400° C. The radioactivity of the ashes was measured with an ionization chamber. All measurements were calibrated against a uranium standard and the activities were corrected for rate of decay of radiophosphorus (half-life 14.3 days) to date of death. The total phosphorus content (P^{31}) of the ashes was determined by Pregl's method. By dividing the amount of radioactivity (microcuries) per gm. wet weight of each tissue by the number of mgm. of phosphorus (P^{31}) per gm. wet weight of the same tissue, there is obtained the "specific radioactivity" of the tissue in μc./mgm. P^{31}.

The tissues so analyzed were cerebellum, kidney, liver, lung, lymph nodes, heart and skeletal muscle, spleen, and sternum. The specific radioactivity of these tissues was independent of the variations in type of disease, age, quantity or route of administration of radiophosphorus, quantity of ingested nonradioactive phosphorus, and quantity of P^{31} and P^{32} per gm. of a specific tissue; but was apparently dependent upon the interval between the last dose of radiophosphorus and death.

Fairly large amounts of P^{32} are retained by the tissues at the end of the 2nd day after administration; however a large part of this quantity is lost between the 2nd and 11th day. That retained appears to be specific for each type of tissue.—M. B.

ERF, L. A., and J. H. LAWRENCE. [Univ. of California, Berkeley, Calif.] PHOSPHORUS METABOLISM IN NEOPLASTIC TISSUE. Proc. Soc. Exper. Biol. & Med., 46:694-695. 1941.

The phosphorus was prepared by bombardment of red phosphorus with deuterons in the cyclotron with subsequent conversion into a neutral, isotonic solution of sodium phosphate which was given orally. The tissues assayed were obtained at the surgical and autopsy tables from patients given the radiophosphate shortly before death. After weighing, each sample was ashed at 450° C. and the radioactivity measured by means of a Lauritsen microscope. The tissues were almost always obtained between the 5th and 9th days following the last administration of radiophosphorus.

In cases with seminoma, melanoma, and fibrosarcoma, the malignant tissues or those infiltrated with malignant cells contained considerably more radiophosphorus than the other (normal) tissues. In cases of neuroblastoma, Ewing's sarcoma, and one active Hodgkin's disease, the abnormal tissues contained as much radiophosphorus as did normal liver and kidney. One case of Hodgkin's disease with densely fibrosed nodes and one lymphosarcoma contained less P^{32} than most other tissues assayed.—M. B.

KOHMAN, T. P., and H. P. RUSCH. [Univ. of Wisconsin, Madison, Wis.] RELATED METABOLIC ACTIVITIES OF NORMAL AND TUMOROUS LIVER NUCLEOPROTEINS INDICATED BY RADIOPHOSPHORUS. Proc. Soc. Exper. Biol. & Med., 46:403-404. 1941.

Experiment I. Of 3 groups of stock albino rats group I received a semisynthetic basal diet consisting of dextrin 79, casein 10, butter 5, salts 4, yeast 1, cod liver oil 1; group II the basal diet plus 0.06% dimethylaminoazobenzene and group III the basal diet, the azo dye, and 10% whole dried liver.

Experiment II. Of 2 groups of stock albino mice, group I received a stock diet (after Kinosita) and group II the stock diet plus 0.05% p-aminoazotoluene. The rats were kept on the diet for 5 months and the mice for 7 months before the experiments, at which time all the animals receiving the azo dyes but no liver had developed hepatomas.

Each animal received subcutaneously 0.5 cc. of a neutral radiophosphate solution. After 24 hours the animals were killed and the livers extracted 2 to 3 times with cold 0.01-2N NH_4OH. The entire protein fraction was precipitated with cold trichloracetic acid and the precipitate washed several times each with cold dilute trichloracetic acid, acetone, alcohol at 70° C., and ether, in order to remove all non-nucleic phosphorus. The residue was then wet-ashed, the total phosphorus measured by colorimeter and the radioactivity measured with a Geiger-Muller counter.

The results showed that the tumorous livers exhibited an increased uptake of labeled phosphate in the nucleoprotein fraction compared to the controls. This increase amounted to $44 \pm 11\%$ for the rats and $46 \pm 7\%$ for the mice. These experiments indicate that the nucleoprotein metabolism is increased in neoplastic tissue.—M. B.

MILLER, J. A., D. L. MINER, H. P. RUSCH, and C. A. BAUMANN. [Univ. of Wisconsin, Madison, Wis.] DIET AND

HEPATIC TUMOR FORMATION. Cancer Research, 1:699-708. 1941.

Liver tumors were produced in rats by feeding butter-yellow (N,N-dimethyl-p-aminoazobenzene) in semi-synthetic diets of controllable inadequacy for 4 months followed by the basal dye-free diet for 2 months or more. The livers were inspected by laparotomy at 4 and 6 months. A tumor incidence of 90 to 100% in 4 months was observed on diets containing 10 to 12% casein but adequate in all other respects.

High levels of dietary casein offered partial protection against butter-yellow. Other protein sources as liver, yeast, and egg offered protection at lower levels of total protein. Whole dried beef liver at 10 to 20% levels offered nearly complete protection at 4 and 6 months. When the dye was fed continuously in a diet containing liver, some tumors eventually developed.

The water-soluble, alcohol-insoluble portion of whole liver was found to offer nearly complete protection at 4 months when fed at low levels in the diet. However, protection was incomplete at 6 months. Xanthine, l-cystine, i-inositol, and choline at 0.1 to 0.5 per cent levels did not offer any protection against the dye.

Butter-yellow reduced the food consumption and growth rate in young rats. Controlled feeding experiments indicated that the dye itself reduced growth *per se* as well as through a decreased food intake.

Results with 34 diets indicated that in general (a) the nutritionally adequate diets offered at least partial protection against hepatoma formation; (b) the protective supplements were usually rich in both protein and the vitamin B complex, particularly riboflavin; (c) the non-protective diets were deficient in at least one of these factors.—Authors' abstract.

WEIL, L. [Biochem. Res. Found., Franklin Inst., Newark, Del.] STUDIES ON PLASMA PHOSPHATASE ACTIVITY DURING EMBRYONIC AND TUMOR GROWTH. J. Biol. Chem., 138:375-380. 1941.

Although a similarity existed between embryonic and tumor growth with regard to the plasma proteinase activity of albino rats, no such similarity was found in the case of plasma phosphatase activity. Rats with Jensen sarcoma did not show any change in plasma phosphatase activity during tumor growth or during the regression of the sarcoma whereas marked enzymatic changes occurred during embryonic growth.—H. J. C.

WEST, P. M., and W. H. WOGLOM. [Dept. Cancer Research, Columbia Univ.] THE BIOTIN CONTENT OF TUMOR AND OTHER TISSUES. Science, 93:525-527. 1941.

Assays for biotin (vitamin H) were made on acid hydrolyzed tissues by a microbiological method based on the specific requirement of *Rhizobium trifolii* (Wisconsin strain 205) for this factor. Results are expressed in terms of pure biotin by standardization of the method with the crystalline methyl ester. Biotin levels from the same organs of adult Wistar rats fed laboratory stock ration were very uniform although individual organs of the same animal differed widely. Expressed in microgammas of biotin per gm. of dried tissue, adult rat skin, muscle, lung, and brain contained much less than heart, kidney, or liver. In the corresponding embryo tissues, however, the reverse was found to be true.

Human carcinoma of sigmoid, rectum, and lung were found distinctly richer in growth substance than adjacent normal tissue. The skin of wild and domestic adult rabbits was found to have less biotin than the Shope papilloma or rabbit embryo skin. Primary carcinoma of the rat liver induced by feeding butter yellow was decidedly poorer in biotin than the tissue adjacent to the neoplasm, unlike the other tumors examined.

The writers believe that the increased biotin content of tumor and embryo represents a difference in metabolism shared by these tissues, rather than a change common to tissues in which cells are rapidly dividing, since the biotin content of vigorously regenerating rat liver or of pregnant human uterus does not differ from the same tissues in a nonhyperplastic state.—M. B.

WOODWARD, G. E., F. E. REINHART, and J. S. DOHAN. [Biochem. Res. Found., Franklin Inst., Newark, Del.] THE GLUTAMIC ACID OF MALIGNANT TISSUE PROTEINS. J. Biol. Chem., 138:677-688. 1941.

Using the procedures of Kögl and Erxleben, the authors were able to isolate glutamic acid hydrochloride from several types of tumors in 50-70% yield. The d(-)-glutamic acid content of the total hydrochloride isolated from any one tissue did not exceed 4.4%; the average value was 2.5%. These results, therefore, do not support the claim that the glutamic acid of malignant tissues exists in a highly racemized state.—H. J. C.

Clinical and Pathological Reports

Nervous System

BAILY, P. [Univ. of Illinois, Chicago, Ill.] INDICATIONS FOR SURGICAL TREATMENT OF INTRACRANIAL TUMOR. South. Surgeon, 9:539-551. 1940.

The author makes a plea for two cardinal principles in surgical methods for the eradication of intracranial neoplasms: a guiding knowledge of the pathology of tumors and an estimation of the functional importance of the operative area. Consistently radical surgery, which may produce some permanent tumor excisions but result in a vegetative existence for the survivor, is not advocated. Tumors of the bulb and pons are always inoperable. Hypophyseal growths are generally amenable to radiation. Meningioma and acoustic nerve neurinoma as well as cerebellar astrocytoma, oligodendroglioma, or ependymoma are relatively benign growths and may be totally extirpated with a chance for a permanent cure. It may be justifiable to remove considerable brain tissue to gain access to a benign tumor. However, for malignant growths such as medulloblastoma and glioblastoma only palliative decompression and roentgen therapy are advised.—M. J. E.

BENDER, M. B., and M. G. KANZER. [Mt. Sinai Hosp., New York, N. Y.] METAMORPHOPSIA AND OTHER

PSYCHOVISUAL DISTURBANCES IN A PATIENT WITH TUMOR OF THE BRAIN. Arch. Neurol. and Psychiat., 45: 481-485. 1941.

Various forms of visual hallucinations are described in a patient with an inoperable glioma involving the greater portion of the right cerebral hemisphere.—M. J. E.

BRADFORD, F. K. [Houston, Tex.] SPINAL MENINGIOMAS. A REPORT OF 4 CASES, 3 OF WHICH WERE IN NEGROES. South. Surgeon, 9:722-732. 1940.

Pronounced symptomatic improvement occurred in 3 patients following resection of meningioma of the thoracic portion of the spinal cord. A fourth died 6 weeks postoperatively of unspecified cause.—M. J. E.

CHILDS, A. E. [McGill Univ., Montreal Canada] CALCIFICATION OF THE CHOROID PLEXUS AND ITS DISPLACEMENT BY EXPANDING INTRACRANIAL LESIONS. Am. J. Roentgenol., 45:523-536. 1941.

In 1,000 consecutive roentgen examinations of the skull, calcification of the choroid plexus was found in 112 cases, or 11.2%. In 6 of 8 cases showing displacement of the calcified glomus the lesions were rapidly growing (4 glioblastoma multiforme, 1 metastatic carcinoma, and 1 hemorrhage). Two were meningeal fibroblastomas.—E. A. L.

COACHMAN, E. H. [Muskogee, Okla.] BRAIN TUMORS I'VE MET. J. Oklahoma M. A., 33:7-9. 1940.

The importance of recognizing intracranial lesions in patients observed by the otolaryngologist and ophthalmologist for symptoms commonly treated in these specialized practices is illustrated by 3 case reports. In each instance more careful examination disclosed evidence of an expanding process in the brain.—M. J. E.

CRAIG, W. McK., and C. H SHELDEN. [Mayo Clinic, Rochester, Minn.] TUMORS OF THE CERVICAL PORTION OF THE SPINAL CORD. Arch. Neurol. & Psychiat., 44:1-16. 1940.

The clinical findings are reviewed in 91 verified cases of tumor of the cervical cord. Morphologically the most common extradural tumor was neurofibroma; intradural and extramedullary tumor, meningioma or neurofibroma; intramedullary tumor, glioma. Typical cases are described and drawings illustrate the surgical technic.—M. J. E.

DAVIDOFF, L. M. [Brooklyn, N. Y.] A THIRTEEN YEAR FOLLOW-UP STUDY OF A SERIES OF CASES OF VERIFIED TUMORS OF THE BRAIN. Arch. Neurol. & Psychiat., 44: 1246-1261. 1940.

A summary is given of the late results of surgical treatment of intracranial tumors operated on in Cushing's clinic during 1925 to 1926. In cases of malignant glial tumors survivals are rare. A considerable number of permanent cures 7 to 11 years after operation was observed in cases of more benign neoplasms as ependymoma, papilloma of the choroid plexus, pituitary adenoma, meningioma, acoustic nerve neurinoma, and cranio pharyngioma. —M. J. E.

DENES, J. [Metropolitan Saint Stephen Hosp., Budapest] A CASE OF INTRACRANIAL CONGENITAL TERATOMA CONSISTING MAINLY OF NERVE ELEMENTS. Am. J. Cancer, 40:329-334. 1940.

A large intracranial teratoma was found in a premature male fetus delivered by cesarian section. The tumor contained bone, cartilage, and glandular structures but was chiefly composed of nerve elements.—L. L. W.

FURRER, E. D., and I. R. FOX. [Eugene, Ore.] PERINEURAL FIBROSARCOMA OF THE LEFT VAGUS SHEATH. REPORT OF A CASE. West. J. Surg., 48:584-586. 1940.

A perineural fibrosarcoma of the cervical portion of the left vagus nerve gave rise to pain in the neck, hoarseness, dysphagia, and a Horner's syndrome of 3 years' duration. The symptoms were originally attributed to a thyroid adenoma, but were not relieved by a thyroidectomy. Radiotherapy had a palliative effect. The patient died of auricular fibrillation. Necropsy disclosed the correct diagnosis and a metastatic focus in the tail of the pancreas. A photograph of the tumor and photomicrographs are included.—M. J. E.

HERREN, R. Y. [Portland, Oregon] PAPILLOMA OF THE CHOROID PLEXUS. Arch. Surg., 42:758-774. 1941.

The author presents a case of papilloma of the choroid plexus. Review of approximately 80 cases in the literature shows that the greatest incidence of the disease is in the first decade of life. In children the tumor is usually found in the lateral ventricles, while in adults it occurs most frequently in the fourth ventricle. The pathology and growth characteristics of the tumor are presented. There are 8 tables, 2 photographs, and 1 drawing.—G. De B.

HIMSON, G. W. [Warren City Hosp., Warren, Ohio] INTRA-ORBITAL TUMOR OF THE OPTIC NERVE; REPORT OF A CASE. Ohio State M. J., 37:451-452. 1941.

The existence of an orbital tumor was assumed in a patient with increasing proptosis, loss of vision, and optic atrophy. The eye of the affected side was enucleated, but histologic examination failed to identify an intraocular neoplasm. Swelling of the orbit increased, and a meningioma extending from the optic foramen was subsequently removed by means of exenteration of the orbit. A small osteoma of the orbital wall was also excised and the patient appeared symptom-free several months later.—M. J. E.

HORRAX, G. [The Lahey Clinic, Boston, Mass.] FAVORABLE TYPES OF BRAIN TUMOR AND THE RESULTS OF THEIR OPERATIVE REMOVAL. New England J. Med., 224:307-315. 1941.

Of 400 brain tumors seen consecutively between 1932 and 1939, 56% were classified as permanently removable. In that group of 224 there was a primary mortality of only 12%. The group included 80 meningiomas, 33 acoustic neuromas, 30 pituitary adenomas, 29 gliomas (mostly cystic astrocytomas of the cerebellum) and a miscellaneous group (52) including angiomas, cholesteatomas, craniopharyngiomas, etc. Of 187 survivors, 27 presented severe disabilities. Of the 224 cases 71% were leading useful lives. The paper is illustrated with 9 figures and has a bibliography of 6 papers.—A. M.

JELSMA, F. [Univ. of Louisville Sch. of Med., Louisville, Ky.] HOUR GLASS TUMORS OF THE CERVICAL SPINE. Am. J. Surg., 52:483-488. 1941.

Report of a case of hour glass tumor, associated with a huge brachial plexus tumor, in a patient with von Recklinghausen's disease.—H. G. W.

McDONALD, C. A., and M. KORB. [Providence, R. I.] BRAIN TUMOR WITH NORMAL BRAIN POTENTIALS. Rhode Island M. J., 23:111-113. 1940.

An astrocytoma in the right cerebral cortex anterior to the motor area gave rise to convulsive seizures and a left hemiplegia. A satisfactory temporary improvement

was achieved by partial extirpation of the growth. Brain potentials had been recorded preoperatively by means of electro-encephalographic examination and found to be normal.—M. J. E.

MEREDITH, J. M. [Univ. of Virginia, Charlottesville, Va.] UNUSUAL TUMORS AND TUMOR-LIKE LESIONS OF THE SPINAL CANAL AND ITS CONTENTS WITH SPECIAL REFERENCE TO PITFALLS IN DIAGNOSIS. Virginia M. Monthly, 67:675-687. 1940.

Nine cases are described. In each instance the symptoms and signs indicated a spinal cord tumor. One patient had a simple arachnoidal cyst in the cervical region. Of the 5 patients with tumors in the dorsal region, 2 had neurofibroma and 1 a meningioma. In the lumbar region 1 patient had an extradural hemangioma and a second a nonneoplastic mass consisting of hypertrophied ligamenta flava and fibrocartilage. The patients in this group were cured by extirpation of the benign lesions. Malignant or potentially malignant processes existed in 3 patients. A benign dorsal neurofibroma recurred as a neurogenic sarcoma 5 months after being excised. The malignant tumor was partially resected and irradiated. Roentgen therapy was administered to a second patient with a Ewing's tumor of the dorsal spine producing the clinical syndrome of cord involvement. The third had an angioblastic meningioma in the lumbar region. Partial removal was possible and was followed by roentgen irradiation. The treatment employed in the latter 3 patients produced temporary symptomatic improvement. Drawings, roentgenograms, and photomicrographs of different tumor types are reproduced.—M. J. E.

MEYER, B. C. [Montefiore Hosp., New York, N. Y.] NEOPLASM OF THE POSTERIOR FOSSA SIMULATING CEREBRAL VASCULAR DISEASE: REPORT OF FIVE CASES WITH REFERENCE TO THE ROLE OF THE MEDULLA IN THE PRODUCTION OF ARTERIAL HYPERTENSION. Arch. Neurol. and Psychiat., 45:468-480. 1941.

Five cases of intracranial tumor situated in the posterior fossa are recorded in which the clinical signs led to an erroneous diagnosis of a lesion of cardiovascular origin, and in which a correct diagnosis was established either at necropsy or operation. In 3 instances the growth was an acoustic nerve neuroma, and in 1 each a basilar meningioma and a hemangioma of the cerebellum. Hypertension, frequently labile, was a common feature, but the patients were in age groups commonly affected by this condition. The onset of symptoms in cases of this type may be apoplectiform, and the true nature of the illness further obscured by acute attacks of cardiovascular or respiratory distress. Some symptoms are undoubtedly the result of arteriorsclerosis, and disseminated neurologic manifestations as pareses or paralyses, visual disturbances, and signs of cerebellar and bulbar disease, as occur in these cases, indicate an intracranial lesion but their localizing character is indefinite. No positive proof is forthcoming that an expanding lesion in the posterior fossa is responsible for the hypertensive manifestations. However, several cases are cited of amyotrophic lateral sclerosis in youthful subjects in whom hypertensive disease appeared related to evidence of bulbar involvement.—M. J. E.

MOERSCH, F. P., W. McK. CRAIG, and J. W. KERNOHAN. [Mayo Clinic, Rochester, Minn.] TUMORS OF THE BRAIN IN AGED PERSONS. Arch. Neurol. and Psychiat., 45:235-245. 1941.

A series of 100 cases of verified brain tumor in individuals of 60 years of age or older is analyzed. These cases represent 8% of the total cerebral neoplasms observed. The symptoms and objective signs did not differ basically from those occurring in younger age groups, but the common incidence of cerebral manifestations following vascular lesions in older patients and senile changes complicates the differential diagnosis. Three histologic types constituted 82% of the total tumors (glioblastoma multiforme, 35 cases; meningioma, 25; acoustic nerve neuroma, 22). The patients are a poor surgical risk. Operation was performed in 86 cases and 37 (43%) patients died postoperatively. Patients with glioblastoma who did not succumb as a result of the intervention survived on the average 4 months after operation, those with meningioma and acoustic nerve tumor, 2½ and 3½ years respectively.—M. J. E.

MORRISON, W. H. [Omaha, Neb.] BILATERAL RETINOBLASTOMA (GLIOMA). PRELIMINARY REPORT OF A CASE. Nebraska M. J., 25:445-449. 1940.

Following diagnosis of bilateral retinoblastoma in a child of 16 months the more extensively involved left eye was enucleated. Fractionated roentgen therapy (10,000 r total dose, 200 kv., filter 0.5 mm. Cu + 1 mm. Al, 3 portals) was administered to the other eye during the succeeding 15 months. The intraocular tumor remained stationary in size and satisfactory vision was retained.—M. J. E.

MUNRO, D., J. E. EDWARDS, and W. O. RUSSELL. [Boston City Hosp., Boston, Mass.] MIXED INTRACRANIAL GLIOMAS. Surg. Gynec. & Obst., 72:787-797. 1941.

About 11% of intracranial gliomatous tumors are of truly mixed type, and a patient with a tumor that contains either glioblastomatous or medulloblastomatous tissue will have, with rare exceptions, a life expectancy of not more than about one year. Therefore surgical therapy of intracranial gliomatous tumors should be conditioned as much by the demonstration by the pathologist during operation of gliomatous and medulloblastomatous tissue, as by the accessibility of the tumor that is to be removed.—H. G. W.

PATTERSON, G. H., and F. M. ANDERSON. [Los Angeles Gen. Hosp., Los Angeles, Calif.] INTRACRANIAL TUMORS OCCURRING IN THREE MEMBERS OF A FAMILY. Bull. Los Angeles Neurol. Soc., 5:218-223. 1940.

Hemangioblastoma occurred in a father and 1 son, and an angioblastic meningioma in the second son. In the case of the father a cerebellar cyst had been drained 10 years prior to the present recurrence of symptoms of cerebellar disease. The patient died postoperatively following an attempted removal of the cerebellar mass. Operation disclosed a cyst of similar nature in 1 son, and drainage likewise relieved symptoms. The meningioma in the second son was located in the parasagittal area. Distinct improvement followed its excision. The author discusses the possibility of the lesions representing phases of Lindau's syndrome.—M. J. E.